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ATTN: BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

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Re: Application of **Hiroaki TAKAYAMA, Katsuhiro KONNO and Toshie FUJISHIMA**
VITAMIN D_3 DERIVATIVE AND ITS PRODUCTION METHOD
Our Reference: Q52816
PCT/JP98/01979, filed April 30, 1998

Dear Sir:

Applicants herewith submit the attached papers for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter I of the Patent Cooperation Treaty. Attached hereto is the application identified above which is a verified translation **of PCT International Application No. PCT/JP98/01979, filed April 30, 1998**, comprising the specification, claims and International Search Report. **The executed Declaration and Power of Attorney and Assignment will be submitted at a later date.**

The Government filing fee is calculated as follows:

Total Claims	2 - 20 =	0 x $18 =	$ 000.00
Independent Claims	1 - 3 =	0 x $78 =	$ 000.00
Base Filing Fee	($840.00)		$ 840.00
Multiple Dep. Claim Fee	($260.00)		$ 000.00
TOTAL FILING FEE			**$ 840.00**

A check for the statutory filing fee of $ **840.00** is attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. 1.16 and 1.17 and any petitions for extension of time under 37 C.F.R. 1.136 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from:

Japanese Patent Application	Filing Date
9-114695	May 2, 1997

Respectfully submitted,
SUGHRUE, MION, ZINN, MACPEAK & SEAS
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DESCRIPTION

Vitamin D_3 Derivative and its Production Method

Technical Field

The present invention relates to a new vitamin D_3 derivative having a methyl group at the 2-position and its production method. More particularly, the invention relates to 1, 25-dihydroxy-2-methylvitamin D3 derivatives useful as a treating agent for osteoporosis and their production methods.

Background Art

Hitherto, it has been well known through patent publications and general scientific literature that an active vitamin D_3 plays an extremely important role as a substance controlling the metabolism of calcium, phosphates, etc., in a living body. Further, it is widely known that various kinds of vitamin D derivatives have been used as treating agents for metabolic disorders of vitamin D including osteoporosis and rickets.

Furthermore, there is a report that the fact in which vitamin D_3 has calcium controlling activity and other various kinds of biological activities found in vitamin D_3 is considered attributable to the occurrence of various kinds of selectivity based on the difference between a binding affinity to a vitamin D receptor and that to a vitamin D binding protein.

As a known 2-position substituted vitamin D_3 derivative, 1, 25-dihydroxyvitamin D_3 derivatives, which have a hydroxyl group of α-configuration at the 1-position and a substituent (no substituent, a C_1 to C_6 linear alkyl group substituted with a hydroxyl group at the terminal, a C_1 to C_6 linear alkyloxy group substituted with a hydroxyl group at the terminal, a C_1 to C_5 alkenyl group or a hydroxyl group) of β-configuration at the 2-position, have been reported [Kobayashi, et al., 116th (1996) Congress of The Pharmaceutical Society of Japan, Abstract 3, 88].

Further, 1, 25-dihydroxyvitamin D_3 derivatives having a hydroxyl group of α-configuration at the 1-position and a substituent (3-hydroxypropyl group or 3-fluoropropyl group) of α-configuration at the 2-position have been

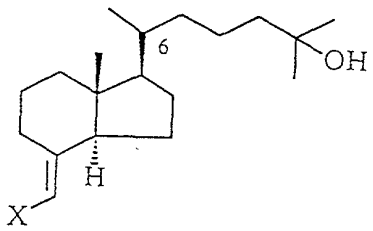
reported [Posner, G. H., J. Org. Chem., 60, 4617 (1995)].

Furthermore, there is a report of a study concerning other stereoisomers based on asymmetric carbons at the 1-, 2- and 3-positions of 1, 25-dihydroxy-2-methylvitamin D3 derivatives (Maki, et al., 116th (1996) Congress of The Pharmaceutical Society of Japan, Abstract 2, 9).

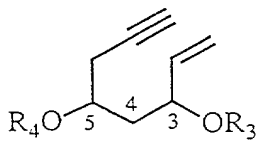
However, no stereoisomer (20S-form) that is different from natural products regarding the configuration of the carbon atom at the 20-position in 1, 25-dihydroxy-2-methylvitamin D_3 derivatives being known, there in no information what kinds of influences does the configuration of the carbon atom at the 20-position exert upon a binding affinity to a vitamin D receptor or to a vitamin D bonding protein, or upon other various kinds of above-mentioned biological activities.

In addition, although methods for producing 2-position substituted vitamin D_3 derivatives are also described in the above reports, only isomers having specific combinations of configurations of the 1-, 2- and 3-asymmetric carbons among all the combinations of the configurations are disclosed in these reports, and no method for efficiently producing an isomer having an arbitrary combination of the configurations is reported.

Recently, a new method through which an active type of vitamin D_3 is synthesized by reacting an exo-methylene compound expressed by the following general formula (II'),



(wherein X is a bromine atom or an iodine atom) with an ene-yne compound expressed by the following general formula (III'),

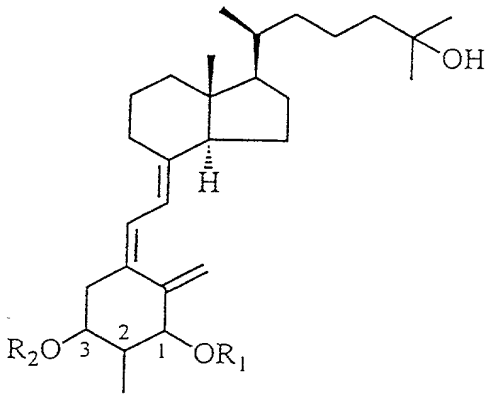


[wherein R_3 and R_4 are each independently a hydrogen atom or a tri(C_1 to C_7 hydrocarbon)silyl group] has been reported [Trost, B. M., J. Am. Chem. Soc., 114, 9836 (1992)]. However, no one has reported that an ene-yne compound having a substituent such as methyl group at the 4-position is used as the above ene-yne compound.

Disclosure of Invention

It is an object of the present invention to provide a new 1, 25-dihydroxy-2-methylvitamin D_3 derivative having biological activity and its production method.

According to the present invention, the first object described above of the present invention can be achieved firstly by a 1, 25-dihydroxy-2-methylvitamin D_3 derivative expressed by the following general formula (I),



[wherein each of R_1 and R_2 is independently a hydrogen atom or a tri(C_1 to C_7 alkyl)silyl group; herein configurations of asymmetric carbons at the 1-, 2- and 3-positions are each independently an α-configuration or a β-configuration].

Namely, the vitamin D_3 derivatives of the present invention include all of the following 8 kinds of derivatives having the configurations at the 1-, 2- and 3-positions of,

(1) the combination of α-configuration, α-configuration and α- configuration,

(2) the combination of α-configuration, α-configuration and β- configuration,

(3) the combination of α-configuration, β-configuration and α- configuration,

(4) the combination of α-configuration, β-configuration and β- configuration,

(5) the combination of β-configuration, α-configuration and α- configuration,

(6) the combination of β-configuration, α-configuration and β- configuration,

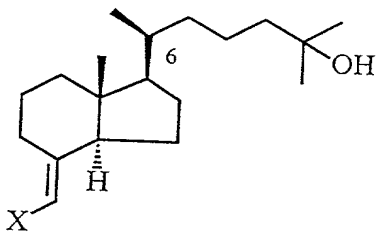
(7) the combination of β-configuration, β-configuration and α- configuration, and

(8) the combination of β-configuration, β-configuration and β-configu-

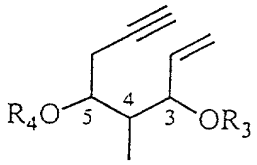
ration. Further, a mixture containing any plural isomers out of the 8 stereoisomers at arbitrary ratios is also included in the derivatives of the present invention.

Further, the notation of the configurations used here for vitamin D analogues follows a usual practice. That is, "α-configuration" used at the 1-, 2- and 3-positions means a configuration containing a bond directing the carbon atom from the upper side of the paper and the "β-configuration" means a configuration containing a bond directing the carbon atom from the lower side of the paper.

In addition, according to the present invention, the above -mentioned object of the present invention is achieved secondly by a method for producing a vitamin D_3 derivative expressed by the above formula (I). That is, a 1, 25-dihydroxy-2-methylvitamin D_3 derivative expressed by the above formula (I) is produced by reacting an exo-methylene compound expressed by the following general formula (II),



(wherein X is a bromine atom or an iodine atom) with an ene-yne compound expressed by the following general formula (III),



[wherein R_3 and R_4 are each independently a hydrogen atom or a tri(C_1 to C_7 hydrocarbon)silyl group] in the presence of a palladium catalyst and optionally removing the protecting group of the tri(C_1 to C_7 hydrocarbon)silyl group.

Best Mode for Carrying out the Invention

In the present invention, the tri(C_1 to C_7 alkyl)silyl groups are each a silyl group substituted with independent three straight or branched C_1 to C_7 alkyl groups, especially preferably a trimethylsilyl, triethylsilyl or t-butyldimethylsilyl group.

Preferred concrete examples of 1, 25-dihydroxy-2-methyl-vitamin D_3 derivatives expressed by the above formula (I) include

(20S)-1α,25-dihydroxy-2β-methyl-3β-vitamin D_3 (68),

(20S)-1β,25-dihydroxy-2β-methyl-3β-vitamin D_3 (69),

(20S)-1α,25-dihydroxy-2β-methyl-3α-vitamin D_3 (70),

(20S)-1β,25-dihydroxy-2β-methyl-3α-vitamin D_3 (71),

(20S)-1α,25-dihydroxy-2α-methyl-3β-vitamin D_3 (72),

(20S)-1β,25-dihydroxy-2α-methyl-3β-vitamin D_3 (73),

(20S)-1α,25-dihydroxy-2α-methyl-3α-vitamin D_3 (74),

(20S)-1β,25-dihydroxy-2α-methyl-3α-vitamin D_3 (75)

(20S)-1α,25-dihydroxy-2β-methyl-3β-vitamin D_3-1,3-bis(trimethylsilyl)ether (76),

(20S)-1β,25-dihydroxy-2β-methyl-3β-vitamin D_3-1,3-bis(trimethylsilyl)ether (77),

(20S)-1α,25-dihydroxy-2β-methyl-3α-vitamin D_3-1,3-bis(trimethylsilyl)ether (78),

(20S)-1β,25-dihydroxy-2β-methyl-3α-vitamin D_3-1,3-bis(trimethylsilyl)ether (79),

(20S)-1α,25-dihydroxy-2α-methyl-3β-vitamin D_3-1,3-bis(trimethylsilyl)ether (80),

(20S)-1β,25-dihydroxy-2α-methyl-3β-vitamin D_3-1,3-bis(trimethylsilyl)ether (81),

(20S)-1α,25-dihydroxy-2α-methyl-3α-vitamin D_3-1,3-bis(trimethylsilyl)ether (82),

(20S)-1β,25-dihydroxy-2α-methyl-3α-vitamin D_3-1,3-bis(trimethylsilyl)ether (83)

(20S)-1α,25-dihydroxy-2β-methyl-3β-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (84),

(20S)-1β,25-dihydroxy-2β-methyl-3β-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (85),

(20S)-1α,25-dihydroxy-2β-methyl-3α-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (86),

(20S)-1β,25-dihydroxy-2β-methyl-3α-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (87),

(20S)-1α,25-dihydroxy-2α-methyl-3β-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (88),

(20S)-1β,25-dihydroxy-2α-methyl-3β-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (89),

(20S)-1α,25-dihydroxy-2α-methyl-3α-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (90), and

(20S)-1β,25-dihydroxy-2α-methyl-3α-vitamin D_3-1,3-bis(t-butyldimethylsilyl)ether (91).

Further, in a method for producing a vitamin D_3 derivative expressed by the above formula (I), an ene-yne compound, a starting material, expressed by the above formula (III) may be selected from all the stereoisomers derived from asymmetric carbons at the 3-, 4- and 5-positions, and mixtures of them at arbitrary ratios. Their configurations are held unchanged during reactions, and a 1, 25-dihydroxy-2-methylvitamin D_3 derivative having the configuration corresponding to the starting material is produced.

A palladium catalyst used in the production method is obtained by combining a zero- or di-valent organic palladium compound with a trisubstituted phosphorus compound. Examples of the organic palladium compound include tetrakis(triphenylphosphine)palladium, tris(dibenzilideneacetone)palladium, tris(dibenzilideneacetone)-palladium-chloroform adduct and palladium acetate. Further, examples of the trisubstituted phosphorous compound include triphenylphosphine and tributylphosphine. As an example of a palladium catalyst prepared by combining both the components, a catalyst having the combination of tris(dibenzilideneacetone)palladium

and triphenylphosphine or that of tris(dibenzilideneacetone)palladium-chloroform adduct and triphenylphosphine is preferably cited, and the mixing ratio is preferably (1:1) to (1:10).

Here, the molar ratio of an exo-methylene compound expressed by the above formula (II) to an ene-yne compound expressed by the above formula (III) is preferably in the range from (1:5) to (5:1), and further a palladium catalyst is used in the range of 0.1-100 mol%, preferably 1-20 mol% based on the exo-methylene compound.

Further, as a reaction solvent used in the reaction of an exo-methylene compound expressed by the above formula (II) with an ene-yne compound expressed by the above formula (III), a nonpolar solvent such as hexane, heptane or toluene, a polar solvent such as diethyl ether, tetrahydrofuran, dioxane, dimethoxyethane, N,N-dimethylformamide or acetonitrile, or a mixture of such solvents is cited, and heptane or toluene is preferred among them. Furthermore, when used for the reaction, the solvent is preferably subjected to a pretreatment such as distillation and/or nitrogen displacement, and the reaction is carried out at a temperature in the range from ambient temperature to the boiling point of the solvent.

Further, in order to trap an acid such as a hydrogen halide to be formed in the reaction system, it is preferable to carry out the reaction in the presence of a base e.g. triethylamine or diisopropylethylamine. The amount of the base to be added is preferably one equivalent or more based on the component which is used in a larger equivalent than the other between the reactants expressed by the above formulae (II) and (III).

Among vitamin D_3 derivatives of the above formula (I) to be obtained in the above reaction, a compound whose R_1 and R_2 are each a tri(C_1-C_7 alkyl)silyl group can be converted into a compound whose R_1 and R_2 are each H by optionally carrying out deprotection reaction.

Such deprotection reaction may be carried out according to a method known per se [for example, Calverley, M. J., Tetrahedron, 43, 4609 (1987); Ho, P. T., Tetrahedron, Letters 1623 (1978)]. Examples of the cleavage agent used here include tetrabutylammonium fluoride, lithium tetrafluoroborate, pyridinium p-toluenesulfonate or camphorsulfonic acid.

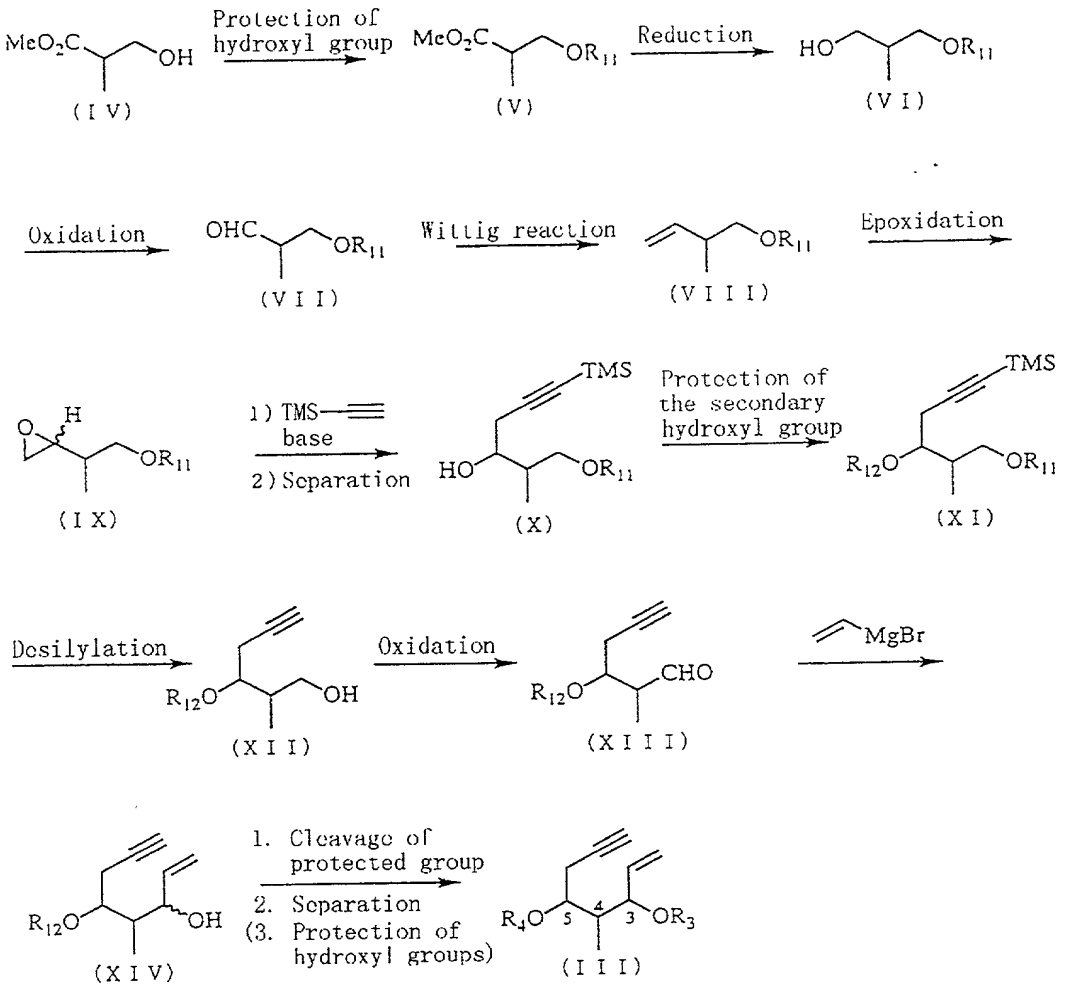
Further, an exo-methylene compound expressed by the above formula

(II) can be synthesized according to a method known per se (B. Fernandez, et al., J. Org. Chem., 1992, 57, 3173; M. J. Calverley, et al., Chem. Lett., 1993, 3, 1845; A. Kutner, et al., J. Org. Chem., 1988, 53, 3450).

Preferred concrete examples of an ene-yne compound, which are used in the production method of the present invention, expressed by the above formula (III) include

(3R,4R,5R)-3,5-dihydroxy-4-methyl-1-octen-7-yne (22),
3S,4R,5R)-3,5-dihydroxy-4-methyl-1-octen-7-yne (23),
(3R,4R,5S)-3,5-dihydroxy-4-methyl-1-octen-7-yne (24),
(3S,4R,5S)-3,5-dihydroxy-4-methyl-1-octen-7-yne (25),
(3R,4S,5R)-3,5-dihydroxy-4-methyl-1-octen-7-yne (26),
(3S,4S,5R)-3,5-dihydroxy-4-methyl-1-octen-7-yne (27),
(3R,4S,5S)-3,5-dihydroxy-4-methyl-1-octen-7-yne (28),
(3S,4S,5S)-3,5-dihydroxy-4-methyl-1-octen-7-yne (29),
(3R,4R,5R)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (30),
(3S,4R,5R)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (31),
(3R,4R,5S)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (32),
(3S,4R,5S)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (33),
(3R,4S,5R)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (34),
(3S,4S,5R)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (35),
(3R,4S,5S)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (36),
(3S,4S,5S)-3,5-bis(trimethylsilyloxy)-4-methyl-1-octen-7-yne (37),
(3R,4R,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (38),
(3S,4R,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (39),
(3R,4R,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (40),
(3S,4R,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (41),
(3R,4S,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (42),
(3S,4S,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (43),
(3R,4S,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (44) and
(3S,4S,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (45).

An ene-yne compound, which is expressed by the above formula (III) and used in the production method of the present invention, can be synthesized, for example, by the following Scheme 1.



Scheme 1

In the above scheme 1, R_{11} is a tri(C_1 to C_7 alkyl)silyl group or a (C_1 to C_7 alkyl)di(C_6 to C_{10} aryl)silyl group, and preferred examples of R_{11} include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl and t-butyldiphenylsilyl groups. Further, R_{12} is a protecting group which together with an oxygen atom bonded to the R_{12} forms an acetal, and methoxymethyl, methoxyethoxymethyl and tetrahydropyranyl groups are suitable as the R_{12}.

This production is carried out as follows. At first, the hydroxyl group of a commercially available optically active ester compound (IV) is protected by a silyl group in the presence of a base to obtain a compound (V). As the

silylating agent used here, triethylsilyl chloride, t-butyldimethylsilyl chloride, t-butyldiphenylsilyl chloride, triethylsilyl triflate, t-butyldimethylsilyl triflate, etc., is preferably used. Further, as the base of the reaction, a common base such as triethylamine, 2,6- lutidine or imidazole is used.

Subsequently, the compound (V) is reduced with a hydride-type reducing agent to obtain an alcohol (VI). As the hydride-type reducing agent, lithium aluminum hydride, diisobutylaluminum hydride, etc., is preferable. The produced hydroxyl group is oxidized with dimethyl sulfoxide/oxalyl chloride, TPAP (tetrapropylammonium perruthenate)/N-methylmorpholine-N-oxide, etc., to obtain an aldehyde (VII), and subjecting the obtained aldehyde (VII) to a common Witting reaction to obtain a methylene compound (VIII).

Subsequently, the resultant double bond is oxidized with a peroxide such as hydrogen peroxide or m-chloroperbenzoic acid to obtain an epoxide compound (IX) and, the obtained epoxide compound is made to react with an acetylene derivative shown in the above scheme in the presence of a base such as alkyl lithium to obtain a compound (X). The compound (X) is formed as a (1:1) mixture of a pair of diastereomers based on the stereoisomerism of the hydroxyl group of the compound (X), while these diastereomers can easily be separated and purified by an ordinary separation process such as column chromatography. Further, configurations of the hydroxyl groups of the separated diastereomers can be determined by measuring ^{1}H-NMR after converting the separated diastereomers to MTPA esters of (R)- and (S)-types [Kusumi, et al., Journal of Synthetic Organic Chemistry, JAPAN, 51, 462 (1996)].

Further, each of the separated compounds (X) is subjected to the following reactions to produce an objective ene-yne compound expressed by the above formula (III) in an optically pure state. That is, the hydroxyl group of a compound (X) is protected with an acetal to obtain a compound (XI). As the acetal-forming agent, methoxymethyl chloride, methoxyethoxymethyl chloride, dihydropyran, etc., is used. Then, the compound (XI) is treated with a fluoride agent such as tetrabutylammonium fluoride to give a desilylated compound (XII), and the resultant primary hydroxyl group is oxidized with dimethyl sulfoxide/oxalyl chloride, TPAP (tetrapropylammonium

perruthenate)/N-methylmorpholine-N-oxide, etc., to form an aldehyde (XIII). Further, the aldehyde group is made to react with a vinyl Grignard reagent to obtain a compound (XIV). Finally, the acetal-type protecting group for the hydroxyl group at the 5-position is removed under an acidic condition to obtain the objective ene-yne compound (III).

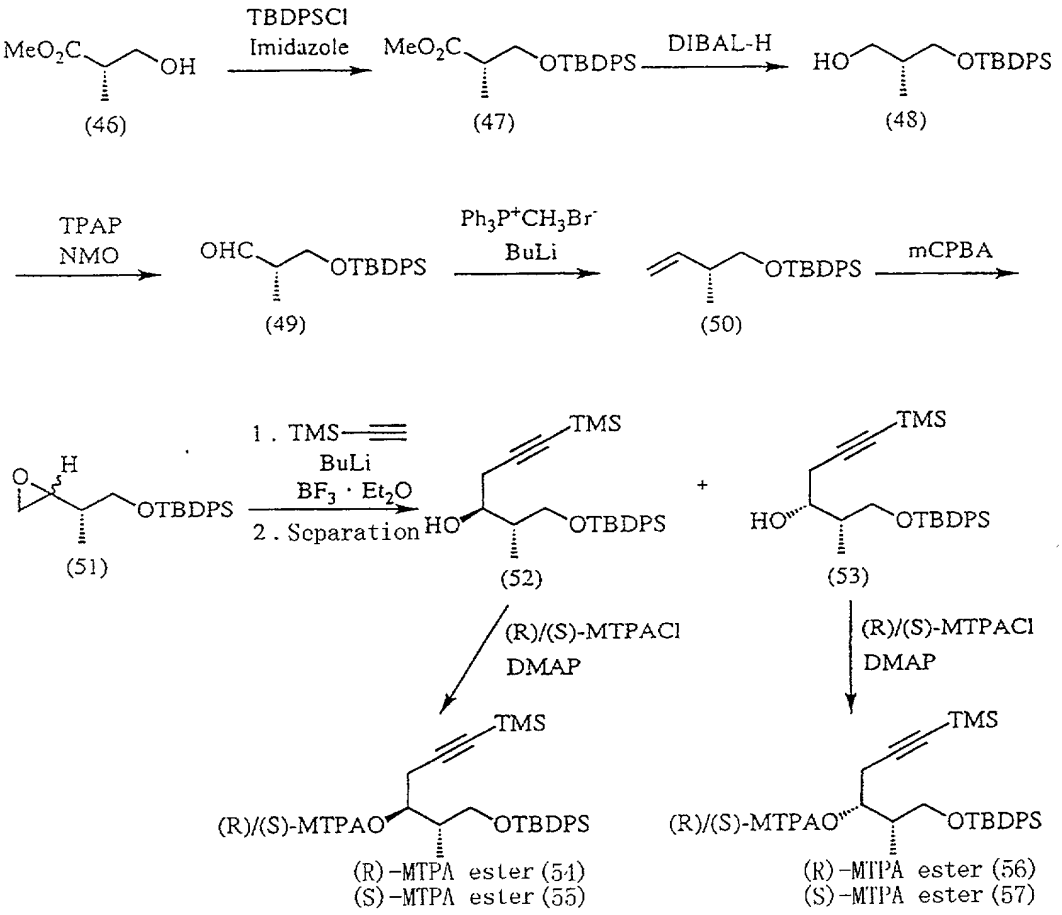
The compound (III) is formed as a (1:1) mixture of two kinds of diastereomers based on the stereoisomerism of the hydroxyl group at the 3-position, while these diastereomers can be easily separated and purified by an ordinary separation process such as column chromatography. Further, the configuration of the hydroxyl group of the separated diastereomers can be determined by measuring ^{13}C-NMR after converting the separated diastereomers to acetonide compounds derived from the 3- and 5-hydroxyl groups [Rychnovsky, S. D., J. Org. Chem., 58, 3511 (1993)]. Furthermore, if necessary, the hydroxyl groups at the 3- and the 5-positions can be protected with silyl groups.

As the silylating agent used here, trimethylsilyl chloride, triethylsilyl chloride, t-butyldimethylsilyl chloride, t-butyldiphenylsilyl chloride, triethylsilyl triflate, t-butyldimethylsilyl triflate, etc., is preferably used, and as the base of the reaction, a common base such as triethylamine, 2,6- lutidine or imidazole is used. The reaction conditions, a solvent, a reaction temperature, etc., of each reaction process in the above reactions are those which are commonly used in such a reaction process.

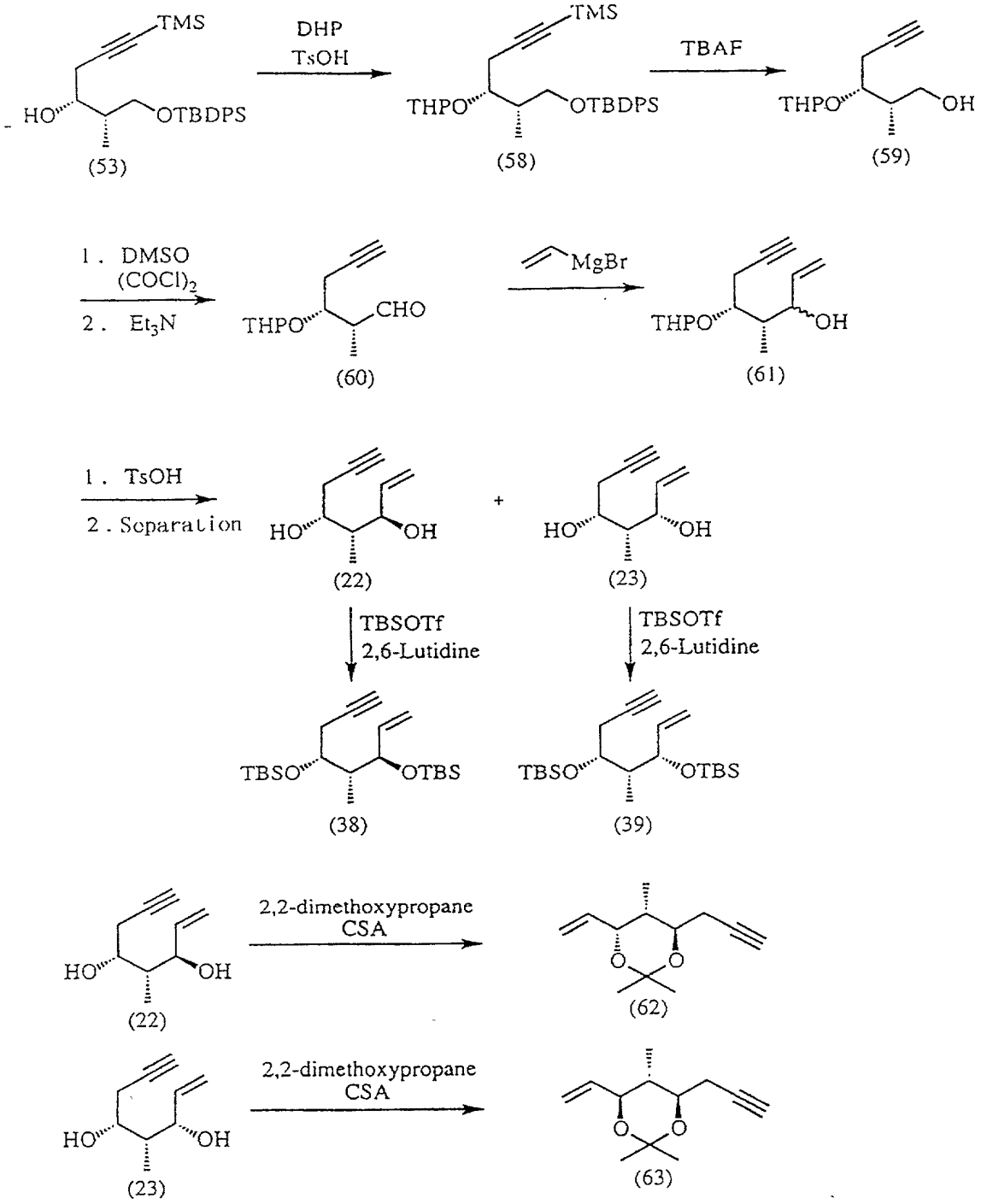
In the production method, the configuration of the methyl group at the 4-position of the objective ene-yne compound (III) is derived from an optically active ester compound (IV) as the starting material (IV), and in the synthetic rout of the present invention, the configuration is held through all the reactions. That is, an important intermediate (III) for the synthesis of vitamins D_3 can be produced in an optically pure state by using an optically active ester compound (IV) as the starting material and consistently adapting reactions capable of holding the configuration.

As examples of the method for producing an optically pure ene-yne compound, synthetic methods for (3R,4R,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (38) and (3S,4R,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (39) are shown by the following schemes 2 and 3,

respectively.



Scheme 2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DHP
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TMS
THPO
OTBDPS
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OH
(22)
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HO
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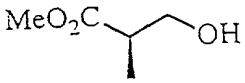
(22)
2,2-dimethoxypropane
CSA
O
O
(62)
HO
OH
(23)
2,2-dimethoxypropane
CSA
O
O
(63)

Scheme 3

[in the above schemes, TBDPSCl is t-butyldiphenylsilyl chloride, DIBAL-H is diisobutylaluminum hydride, TPAP is tetrapropyl-ammonium perruthenate, NMO is N-methylmorpholine-N-oxide, mCPBA is m-chloroperbenzoic acid, MTPACl is α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, DMAP is 4-(dimethyl-amino)pyridine, DHP is dihydropyran, TsOH is p-toluenesulfonic acid, TBAF is tetrabutylammonium fluoride and TBSOTf is t-butyldimethylsilyl triflate, TBDPS is a t-butyldiphenylsilyl group, TBS is a t-butyldimethylsilyl group and THP is a tetrahydropyranyl group].

Besides these examples, e.g. a compound of (4R,5S)-series can be synthesized by a similar production method using a compound (52) obtained through the above scheme 2, and a compound of (4S)-series can be synthesized by a similar production method using the following optically active ester (64), as the starting material respectively.



(64)

Examples

The present invention will be explained further in detail hereafter with examples, while the present invention is not restricted by the examples.

At first, preparations of compounds of the above-mentioned formula (III) , which are synthetic intermediates of compounds of the present invention, are described as reference examples.

Reference Example 1

Synthesis of methyl (S)-3-(t-butyldiphenylsilyloxy)-2-methylpropionate (47)

(46) (47)

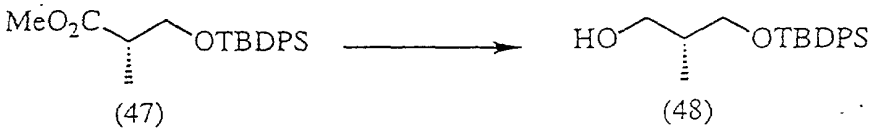
Under argon atmosphere, Methyl (S)-3-hydroxy-2-methyl-propionate (46) (1.9 ml, 2.0 g, 16.9 mmol) was dissolved in 100 ml of dichloromethane, imidazole (2.3 g, 32.5 mmol) and TBDPSCl (4.3 ml, 16.9 mmol) were added to the resultant solution, and the mixture was stirred for 5 min. The reaction mixture was extracted with ethyl acetate after the addition of H_2O. The ethyl acetate layer was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by column chromatography (60 g, 2% AcOEt-hexane) to obtain a colorless oily product (47) (6.5 g, quant.).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 1.04 (9H, s), 1.15 (3H, d, J=7.0 Hz), 2.72 (1H, dquin, J=5.8, 7.0 Hz), 3.67 (3H, s), 3.73 (1H, dd, J=6.4, 9.8 Hz), 3.83 (1H, dd, J=9.8, 6.4 Hz), 7.35-7.44 (6H, m), 7.64-7.68 (4H, m).

MS m/z: 325 (M^+-Me-Me), 299 (M^+-tBu).

Reference Example 2

Synthesis of (R)-3-(t-butyldiphenylsilyloxy)-2-methylpropanol (48)



(47) (48)

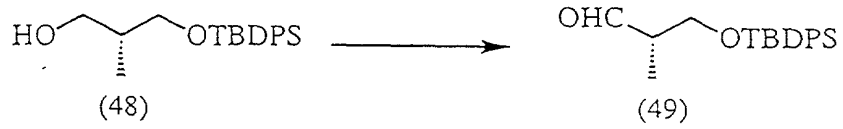
Under argon atmosphere, Methyl (S)-3-(t-butyldiphenylsilyloxy)-2-metylpropionate (47) (1.0 g, 2.7 mmol) was dissolved in 50 ml of dry toluene, 1M DIBAL-H/hexane (5.7 ml, 5.7 mmol) was added to the resultant solution at 0℃, and the mixture was stirred for 15 min. Subsequently, the reaction mixture was further stirred for 45 min after the temperature was returned to room temperature. Ethyl acetate was added to the reaction mixture to decompose the excess DIBAL-H, and the resultant mixture was extracted with 0.5N HCl. The ethyl acetate layer was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by column chromatography (30 g, 4-10% AcOEt-hexane) to obtain a colorless oily product (48) (968 mg, quant.).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.83 (3H, d, J=7.0 Hz), 1.06 (9H, s), 1.99 (1H, ddddq, J=4.6, 5.2, 6.2, 7.0 Hz), 2.58 (1H, bs), 3.60 (1H, dd, J=7.6, 10.1 Hz), 3.97 (2H, d, J=6.4 Hz), 3.72 (1H, dd, J=4.6, 10.1 Hz), 7.37-7.46 (6H, m), 7.67-7.69 (4H, m).

MS m/z: 328 (M^+), 271(M^+-tBu).

Reference Example 3

Synthesis of (S)-3-(t-butyldiphenylsilyloxy)-2-methylpropanal (49)



(48) (49)

Under argon atmosphere, (R)-3-(t-butyldiphenylsilyloxy)-2-

methylpropanol (48) (725 mg, 2.2 mmol) was dissolved in 40 ml of dry dichloromethane. MS-4A (30 mg), NMO (862 mg, 11.1 mmol) and TPAP (catalytic amount) were added to the resultant solution at 0℃, and the mixture was stirred for 15 min. The reaction mixture was further stirred overnight after the temperature was returned to room temperature. Subsequently, H_2O was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The ethyl acetate layer was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by column chromatography (21 g, 4% AcOEt-hexane) to obtain a colorless oily product (49) (700 mg, 97%).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ :1.04 (9H, s), 1.10 (3H, d, J=7.0 Hz), 2.56 (1H, ddddq, J=1.3, 4.8, 6.1, 7.0 Hz), 3.87 (2H, ddd, J=4.8, 6.1, 10.0 Hz), 7.36-7.46 (6H, m), 7.63-7.67 (4H, m), 9.77 (1H, d, J=1.5 Hz).

MS m/z: 325 (M^+-H), 269 (M^+-tBu).

Reference Example 4

Synthesis of (S)-4-(t-butyldiphenylsilyloxy)-3-methyl-1-butene (50)

(49) (50)

Under argon atmosphere, $Ph_3P^+CH_3Br^-$ (2.2 g, 7.4 mmol) was suspended in 15 ml of THF, butyllithium (5.2 ml, 9.3 mmol) was added to the resultant solution at 0℃, and the mixture was stirred for 20 min. The treated mixture was added to a 15-ml THF solution of (S)-3-(t-butyldiphenylsilyloxy)-2-methylpropanal (49) (1.2 g, 3.7 mmol) at 0℃. The resultant mixture was stirred for 15 min and further for 45 min after the temperature was returned to room temperature. Then, the reaction mixture was extracted with ethyl acetate after the addition of a saturated ammonium chloride aqueous solution. The ethyl acetate layer was washed with brine, dried over magnesium sulfate, and evaporated. The crude product was

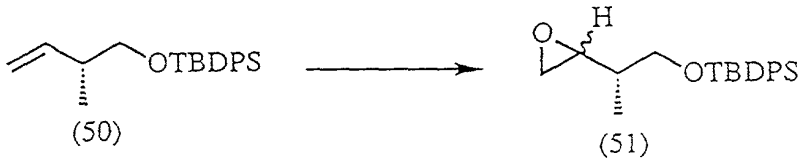
purified by column chromatography (12 g, 2% AcOEt-hexane) to obtain a colorless oily product (50) (1.1 g, 92%).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 1.03 (3H, d, J=7.0 Hz), 1.05 (9H, s), 2.39 (1H, ddq, J=6.0, 6.7, 7.0 Hz), 3.49 (1H, dd, J=6.7, 9.7 Hz), 3.57 (1H, dd, J=6.1, 9.7 Hz), 5.01 (3H, m), 7.35-7.44 (6H, m), 7.65-7.68 (4H, m), 9.77 (1H, d, J=1.5 Hz).

MS m/z: 267 (M^+-tBu).

Reference Example 5

Synthesis of (3S)-4-(t-butyldiphenylsilyloxy)-3-methyl-1-butene oxide (51)



(50) (51)

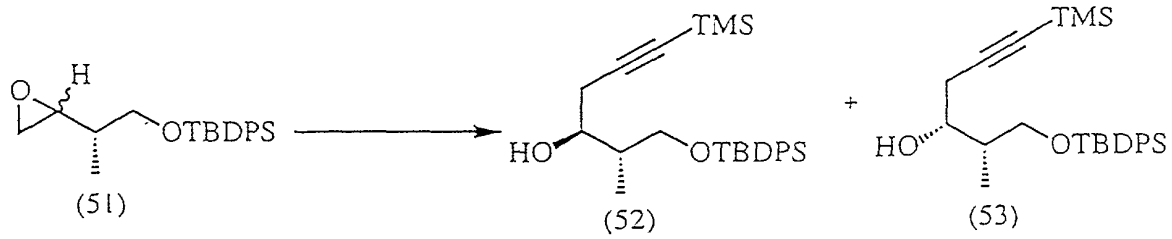
Under argon atmosphere, (S)-4-(t-butyldiphenylsilyloxy)-3-methyl-1-butene (50) (1.0 g, 3.1 mmol) was dissolved in 25 ml of dry dichloromethane, mCPBA (1.4 g, 7.4 mmol) was added to the resultant solution at 0℃, and the mixture was stirred for 15 min. Then, the mixture was further stirred overnight after the temperature was returned to room temperature. The reaction mixture was extracted with ethyl acetate after the addition of water. The ethyl acetate layer was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by column chromatography (30 g, 2% Et_2O-hexane) to obtain a colorless oily product (51). (1.1 g, quant.).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.99 (3H, d, J=6.8 Hz), 1.05 (5H, s), 1.07 (4H, s), 1.58 (1H, dtq, J=5.0, 6.7, 7.0 Hz), 2.57 (4/9H, m), 2.60 (8/9H, dd, J=2.7, 5.0 Hz). 2.73 (4/9H, dd, J=4.3, 5.0 Hz), 2.76 (5/9H, dd, J=4.3, 5.0 Hz), 2.85 (5/9H, ddd, J=2.7, 4.3, 7.0 Hz), 2.97 (4/9H, ddd, J=2.7, 4.3, 7.0 Hz), 3.49 (1H, dd, J=6.7, 9.7 Hz), 3.62 (1H, dd, J=7.0, 9.7 Hz), 3.70 (1H, dd, J=5.0, 9.7 Hz), 4.02 (3H, m), 7.39 (6H, m), 7.67 (4H, m).

MS m/z: 283 (M^+-tBu).

Reference Example 6

Syntheses of (2S,3S)-1-(t-butyldiphenylsilyloxy)-2-methyl-6-tri-methylsilyl-5-hexyn-3-ol (52) and (2S,3R)-1-(t-butyldiphenylsilyl-oxy)-2-methyl-6-trimethylsilyl-5-hexyn-3-ol (53)



Under argon atmosphere, ethynyltrimethylsilane (0.78 ml, 5.0 mmol) was dissolved in 40 ml of THF, and butyllithium (4.5 ml, 5.0 mmol) was added to the resultant solution at 0℃, and the mixture was stirred for 20 min. The treated mixture was cooled to -78℃ and added to 40 ml THF solution of the compound (51) (1.7 g, 5.0 mmol). The resultant mixture was stirred at -78℃ for 15 min after the addition of BF_3·Et_2O (9.5 ml, 5.0 mmol), and further for 2 hr after the temperature was returned to room temperature. The reaction mixture was extracted with ethyl acetate after the addition of a saturated ammonium chloride aqueous solution. The ethyl acetate layer was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by column chromatography (51 g, 2% Et_2O-hexane) to obtain a product (52) (1.2 g, 52%) and a product (53) (1.1 g, 49%), both as colorless oily substances.

52

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.14 (9H, s), 1.00 (3H, d, J=7.0 Hz), 1.06 (9H, s), 1.92-1.99 (1H, m), 2.42 (1H, dd, J=7.0, 10.1 Hz), 2.50 (1H, dd, J=6.7, 10.1 Hz), 2.84 (1H, d, J=3.1 Hz), 3.67 (1H, dd, J=6.4, 10.2 Hz), 3.75 (1H, dd, J=4.2, 10.2 Hz), 3.79 (1H, dd, J=4.3, 10.4 Hz), 7.37-7.46 (6H, m), 7.65-7.68 (4H, m).

MS m/z: 381 (M^+-tBu), 269 (M^+-Me-2Ph), 239 (M^+-2Ph-3Me).

53

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.15 (9H, s), 0.91 (3H, d, J=7.1 Hz), 1.07 (9H, s), 1.93-1.99 (1H, m), 2.46 (1H, dd, J=6.4, 10.6 Hz), 2.54 (1H, dd, J=6.4, 10.6 Hz), 2.84 (1H, d, J=3.1 Hz), 3.67 (1H, dd, J=6.4, 10.4 Hz), 3.74-3.76 (1H, m), 3.79 (1H, dd, J=4.3, 10.4 Hz), 7.37-7.46 (6H, m), 7.65-7.68 (4H, m).

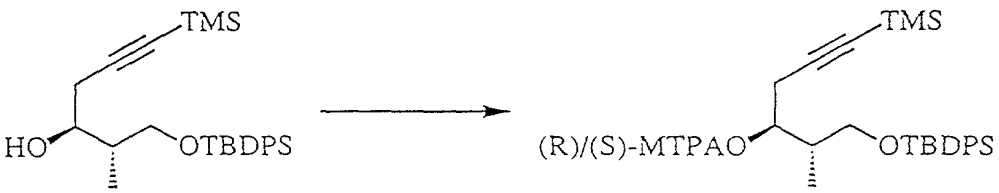
MS m/z : 423 (M^+-Me), 365 (M^+-TMS), 308 (M^+-TMS-tBu).

Reference Example 7

Syntheses of MTPA esters [determination of the absolute configuration of an alcohol (X)]

Under argon atmosphere, each of the above alcohols (X) was dissolved in dry dichloromethane. DMPA (2 equivalent) and (R)- or (S)-MTPACl (2 equivalent) were added to the resultant solution, and the mixture was stirred at room temperature for 4 hr. The reaction mixture was purified on TLC (10% AcOEt-hexane) without having any pretreatment to obtain the corresponding MTPA ester.

Syntheses of compounds (54) and (55) from the compound (52)



(52)

(R)-MTPA ester (54)

(S)-MTPA ester (55)

54(R)

Yield : 30% (colorless oil).

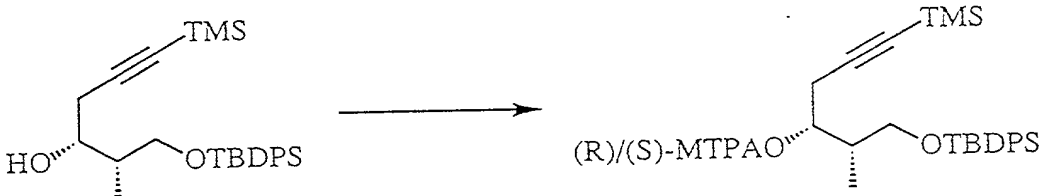
^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.12 (9H, s), 0.80 (3H, d, J=6.7 Hz), 1.06 (9H, s), 2.17 (1H, q, J=6.7 Hz), 2.68 (1H, t, J=6.7 Hz), 3.41 (2H, dd, J=3.0, 10.3 Hz), 3.58 (3H, s), 5.46 (1H, dd, J=6.1, 10.3 Hz), 7.28-7.46 (9H, m), 7.49-7.55 (2H, m), 7.61-7.65 (4H, m).

55(S)

Yield : 25% (colorless oil).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.12 (9H, s), 0.86 (3H, d, J=7.0 Hz), 1.07 (9H, s), 2.28 (1H, q, J=6.1 Hz), 2.57 (1H, dd, J=5.8, 10.6 Hz), 2.71 (1H, dd, J=6.1, 10.6 Hz), 3.46 (3H, s), 3.48 (2H, m), 5.49 (1H, dd, J=5.8, 9.8 Hz), 7.28-7.46 (9H, m), 7.49-7.56 (3H, m), 7.60-7.69 (4H, m).

Syntheses of compounds (56) and (57) from the compound (53)



(53)

(R)-MTPA ester (56)

(S)-MTPA ester (57)

56(R)

Yield: 13% (colorless oil).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.07 (9H, s), 0.95 (3H, d, J=7.0 Hz), 1.05 (9H, s), 2.26 (1H, q, J=6.7 Hz), 2.55 (1H, dd, J=6.1, 11.6 Hz), 2.75 (1H, dd, J=5.2, 11.6 Hz), 3.42 (3H, s), 3.56 (1H, dd, J=5.8, 10.7 Hz), 3.64 (1H, dd, J=6.5, 10.7 Hz), 5.27 (1H, dd, J=5.8, 11.6 Hz), 7.28-7.45 (9H, m), 7.50-7.56 (2H, m), 7.59-7.65 (4H, m).

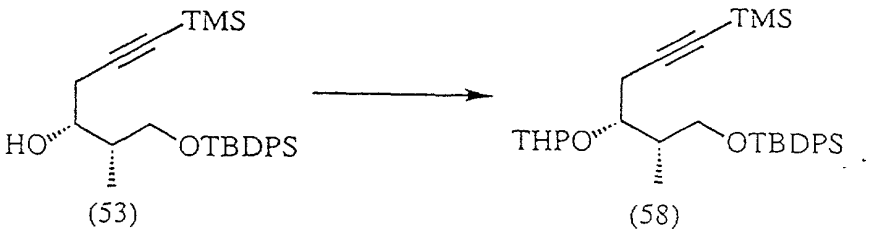
57(S)

Yield: 17% (colorless oil).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.11 (9H, s), 0.82 (3H, d, J=7.0 Hz), 1.05 (9H, s), 2.19 (1H, q, J=6.1 Hz), 2.58 (1H, dd, J=6.7, 11.0 Hz), 2.75 (1H, dd, J=6.7, 11.0 Hz), 3.49 (1H, dd, J=5.4, 10.3 Hz), 3.54 (1H, dd, J=5.8, 10.3 Hz), 3.57 (3H, s), 5.32 (1H, dd, J=6.7, 10.3 Hz), 7.28-7.45 (9H, m), 7.54-7.59 (2H, m) 7.59-7.65 (4H, m).

Reference Example 8

Synthesis of (4R,5S)-6-(t-butyldiphenylsilyloxy)-5-methyl-4-tetrahydropyranyloxy-1-trimethylsilyl-1-hexyne (58)



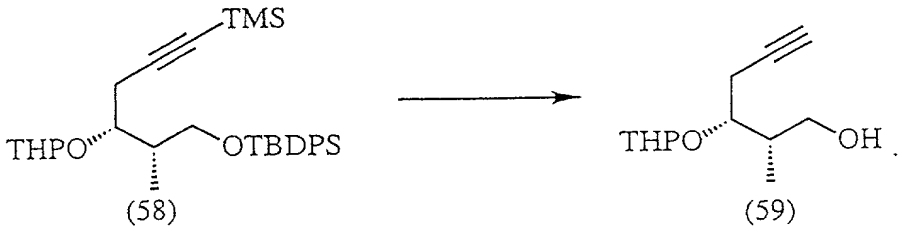
(53) (58)

DHP (0.34 ml, 3.75 mmol, 1.05 equivalent) and TsOH (72 mg, 0.375 mmol, 0.15 equivalent) were added to a dichloromethane solution (10 ml) of the alcohol (53) (1.07 g, 2.50 mmol), and the mixture was allowed to stand overnight at room temperature. A saturated sodium bicarbonate aqueous solution was added to the reaction mixture, and the resultant mixture was extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by silica gel column chromatography (100 g, 1% AcOEt-hexane) to obtain a colorless oily product (58) (1.26 g, 98%).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.127 (9/2H, s), 0.135 (9/2H, s), 0.95 (3H, d, J=7.0 Hz), 1.058 (9/2H, s), 1.061 (9/2H, s), 1.41-1.62 (4H, m), 1.69-1.81 (2H, m), 2.09-2.17 (1H, m), 2.38 (1/2H, dd, J=7.3, 17.1 Hz), 2.46 (1/2H, dd, J=4.6, 17.1 Hz), 2.54 (1/2H, dd, J=5.5, 17.1 Hz), 2.66 (1/2H, dd, J=5.8, 17.1 Hz), 3.38-3.49 (1H, m), 3.58-3.71 (2H, m), 3.75-3.81 (1H, m), 3.88-3.91 (1/2H, m), 3.92-4.06 (1/2H, m), 4.66 (1/2H, dd, J=3.1, 3.4 Hz), 4.86 (1/2H, dd, J=2.7, 4.3 Hz), 7.35-7.44 (6H, m), 7.65-7.70 (4H, m).

Reference Example 9

Synthesis of (2S,3R)-2-methyl-3-tetrahydropyranyloxy-5-hexyn-1-ol (59)



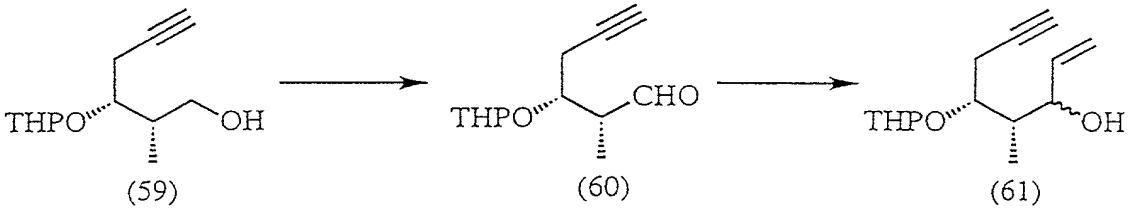
(58) (59)

A 1M nBu_4NF/THF solution (8.8 ml, 8.80 mmol, 4 equivalent) was added to a THF solution (20 ml) of the compound (58) (1.13 g, 2.20 mmol), and the mixture was stirred at room temperature for 4 hr. The reaction mixture was extracted with ethyl acetate after the addition of water. The extract was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by silica gel column chromatography (35 g, 20% AcOEt-hexane) to obtain a colorless oily product (59) (450 mg, 96%).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.99 (3/2H, d, J=6.7 Hz), 1.01 (3/2H, d, J=7.0 Hz), 1.41-1.89 (6H+1/2H, m), 1.99 (1/2H, t, J=2.7 Hz), 2.00 (1/2H, t, J=2.7 Hz), 2.13-2.19 (1/2H, m), 2.33 (1/2H, bs), 2.38 (1/2H, ddd, J=2.4, 6.1, 17.1 Hz), 2.57 (1/2H, ddd, J=2.4, 4.0, 17.1 Hz), 2.63 (1/2H, ddd, J=2.8, 4.0, 17.1 Hz), 2.72 (1/2H, ddd, J=2.8, 7.0, 17.1 Hz), 3.30-3.31 (1/2H, m), 3.41-3.56 (3/2H, m), 3.60-3.81 (2H, m), 3.95-4.01 (3/2H, m), 4.69-4.71 (1H, m).

Reference Example 10

Synthesis of (4R,5R)-3-hydroxy-4-methyl-5-tetrahydropyranyloxy-1-octen-7-yne (61)



(59) (60) (61)

Oxalyl chloride (0.56 ml, 6.30 mmol, 3 equivalent) was added to a dichloromethane solution (4 ml) of DMSO (0.92 ml, 12.5 mmol, 6 equivalent), and the mixture was stirred at -78℃ for 1 hr under argon atmosphere. A

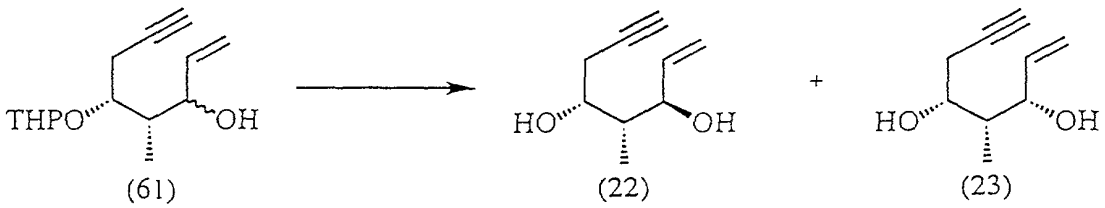
dichloromethane solution (10 ml) of the compound (59) (440 mg, 2.08 mmol) was added to the resultant solution at -78℃, and the mixture was stirred for 30 min. Subsequently, Et_3N (3.2 ml, 24 mmol, 12 equivalent) was added to the mixture followed by stirring for 1 hr, while the temperature was elevated from -78℃ to 0℃. The reaction mixture was extracted with ethyl acetate after the addition of water, the extract was washed with brine, dried over magnesium sulfate and evaporated. The resultant crude product was filtered through a short column of silica gel, and the filtrate was evaporated to obtain a colorless oily aldehyde (60). The product (60) was used for the next reaction without further purification.

A 1M vinyl magnesium bromide/THF solution (4.0 ml, 4.00 mmol, 2 equivalent) was added to a THF solution (10 ml) of the aldehyde (60) (426 mg, 2.02 mmol) at 0℃, the mixture was stirred at 0℃ for 1 hr. The reaction mixture was extracted with ethyl acetate after the addition of water. The extract was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by silica gel column chromatography (55 g, 20% AcOEt-hexane) to obtain a colorless oily allyl alcohol (61) (329 mg, 68%).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.85 (3/4H, d, J=7.0 Hz), 0.88 (3/4H, d, J=7.3 Hz), 0.90 (3/4H, d, J=7.0 Hz), 0.93 (3/4H, d, J=7.0 Hz), 1.47-1.87 (6H, m), 1.98-2.05 (1H, m), 2.15-2.19 (1H, m), 2.37-2.89 (2H, m), 3.37-4.15 (4.5H, m), 4.51-4.84 (1.5H, m), 5.13-5.35 (2H, m), 5.83-5.94 (1H, m).

Reference Example 11

Syntheses of (3R,4R,5R)-3,5-dihydroxy-4-methyl-1-octen-7-yne (22) and (3S,4R,5R)-3,5-dihydroxy-4-methyl-1-octen-7-yne (23)



(61) (22) (23)

TsOH (25 mg, 0.13 mmol, 0.1 equivalent) was added to a methanol solution (10 ml) of the allyl alcohol (61) (315 mg, 1.32 mmol), and the mixture was allowed to stand at room temperature for 1 hr. The reaction mixture was extracted with Et_2O after the addition of a saturated bicarbonate aqueous solution. The extract was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by silica gel column chromatography (55 g, 10% AcOEt-hexane) to obtain a colorless oily ene-yne compound (22) (79 mg, 39%) and a colorless oily ene-yne compound (23) (75 mg, 37%).

22

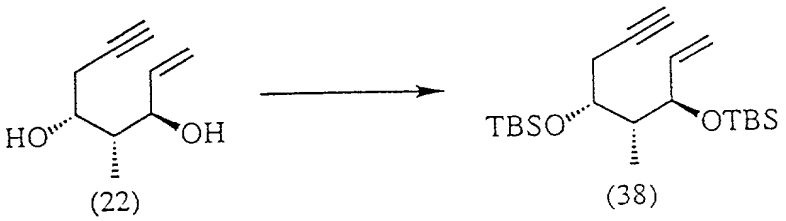
^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.90 (3H, d, J=7.0 Hz), 1.95 (1H, dquin, J=2.8, 7.0 Hz), 2.08 (1H, t, J=2.8 Hz), 2.43 (1H, ddd, J=2.8, 7.0, 17.1 Hz), 2.54 (1H, ddd, J=2.8, 4.6, 17.1 Hz), 2.72 (1H, d, J=5.5 Hz), 2.96 (1H, d, J=4.6 Hz), 3.79 (1H, tt, J=4.6, 7.0 Hz), 4.44 (1H, dtt, J=7.0, 1.5, 5.5 Hz), 5.23 (1H, dt, J=10.7, 1.5 Hz), 5.32 (1H, dt, J=17.1, 1.5 Hz), 5.94 (1H, ddd, J=5.5, 10.7, 17.1 Hz).

23

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.83 (3H, d, J=7.0 Hz), 1.83 (1H, dquin, J=7.0, 7.9 Hz), 2.07 (1H, t, J=2.8 Hz), 2.41 (1H, ddd, J=2.8, 6.7, 16.8 Hz), 2.58 (1H, ddd, J=2.8, 4.0, 16.8 Hz), 2.88 (1H, bs), 3.41 (1H, bs), 3.74 (1H, m), 4.14 (1H, tt, J=1.2, 7.3 Hz), 5.19 (1H, dt, J=10.4, 1.2 Hz), 5.27 (1H, dt, J=17.1, 1.2 Hz), 5.88 (1H, ddd, J=7.3, 10.4, 17.1 Hz).

Reference Example 12

Synthesis of (3R,4R,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (38)



(22) (38)

2,6-Lutidine (0.18 ml, 1.5 mmol, 4 equivalent) and subsequently

TBSOTf (0.34 ml, 1.5 mmol, 4 equivalent) were added to dichloromethane solution (5 ml) of the compound (22) (58 mg, 0.376 mmol), and the mixture was stirred at 0℃ for 1 hr. The reaction mixture was extracted with ethyl acetate after the addition of a saturated bicarbonate aqueous solution. The extract were washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by silica gel column chromatography (10 g, 2% AcOEt-hexane) to obtain a colorless oily ene-yne compound (38) (141 mg, 98%).

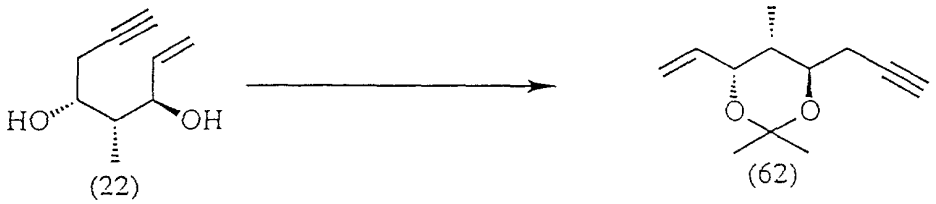
^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.01 (3H, s), 0.5 (3H, s), 0.07 (3H, s), 0.11 (3H, s), 0.89 (9H, s), 0.90 (9H, s), 0.90 (3H, d, J=7.0 Hz), 1.78 (1H, dquin, J=4.9, 7.0 Hz), 1.93 (1H, t, J=2.8 Hz), 2.26 (1H, ddd, J=2.8, 7.0, 16.8 Hz), 2.40 (1H, ddd, J=2.8, 4.3, 16.8 Hz), 3.86 (1H, dt, J=7.0, 4.3 Hz), 4.11 (1H, ddt, J=5.8, 7.3, 1.8 Hz), 5.09 (1H, dt, J=10.1, 1.8 Hz), 5.14 (1H, dt, J=17.4, 1.8 Hz), 5.84 (1H, ddd, J=7.3, 10.1, 17.4 Hz).

Reference Example 13

Syntheses of acetonides [determination of the absolute configuration of an ene-yne compound (III)]

Each (5 mg) of the above ene-yne compounds was dissolved in 0.4 ml of acetone, and the resultant solution was allowed to stand at room temperature for 5 hr after the addition of 0.1 ml of dimethoxypropane and CSA (1.5 mg, 0.2 equivalent). The reaction mixture was evaporated, and the obtained crude product was purified by silica gel column chromatography (6 g, 5% AcOEt-hexane) to obtain an acetonide.

Synthesis of a compound (62) from the compound (22)



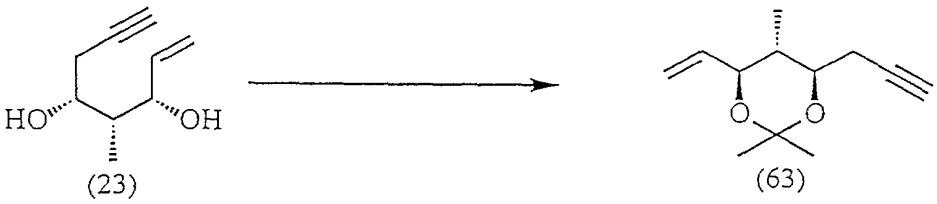
(22) (62)

Yield: 80% (colorless oil).

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.90 (3H, d, J=7.0 Hz), 1.39 (3H, s), 1.40 (3H, s), 1.86-1.92 (1H, m), 2.01 (1H, t, J=2.8 Hz), 2.44 (1H, ddd, J=2.8, 6.1, 17.4 Hz), 2.48 (1H, ddd, J=2.8, 5.5, 17.4 Hz), 3.49 (1H, dt, J=7.6, 5.8 Hz), 4.43 (1H, ddt, J=6.1, 5.2, 1.5 Hz), 5.17 (1H, dt, J=10.7, 1.2 Hz), 5.26 (1H, dt, J=17.4, 1.2 Hz), 5.79 (1H, ddd, J=6.1, 10.7, 17.4 Hz)

^{13}CNMR (100 MHz, $CDCl_3$/TMS) δ : 12.89 (q), 24.10 (q), 25.24 (q), 29.70 (t), 39.76 (d), 69.66 (s), 70.61 (d), 73.02 (d), 80.96 (d), 100.88 (s), 115.77 (t), 135.59 (t).

Synthesis of a compound (63) from the compound (23)



(23) (63)

Yield: 80% (colorless oil).

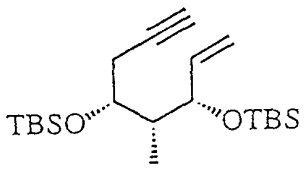
^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.82 (3H, d, J=6.7 Hz), 1.45 (3H, s), 1.49 (3H, s), 1.51-1.61 (1H, m), 2.01 (1H, t, J=2.7 Hz), 2.42 (1H, ddd, J=2.7, 5.5, 17.4 Hz), 2.52 (1H, ddd, J=2.7, 4.0, 17.4 Hz), 3.68 (1H, ddd, J=4.0, 5.8, 10.1 Hz), 3.91 (1H, ddt, J=7.3, 10.1, 1.5 Hz), 5.24 (1H, dd, J=1.5, 7.3 Hz), 5.29 (1H, dd, J=1.5, 17.4 Hz), 5.76 (1H, ddd, J=7.3, 10.1, 17.4 Hz).

^{13}C-NMR (100 MHz, $CDCl_3$/TMS) δ : 12.15 (q), 19.71 (q), 29.70 (t), 30.04 (q), 39.76 (d), 69.66 (s), 70.61 (d), 73.02 (d), 80.96 (d), 100.88 (s), 115.77 (t), 135.59 (t).

Each of the following ene-yne compounds was synthesized by using an appropriate raw material and applying a similar process.

Reference Example 14

Synthesis of (3S,4R,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (39)

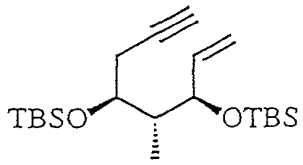


(39)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.02 (3H, s), 0.057 (3H, s), 0.063 (3H, s), 0.11 (3H, s), 0.78 (3H, d, J=7.0 Hz), 0.86 (9H, s), 0.90 (9H, s), 1.89 (1H, dquin, J=5.5, 7.0 Hz), 1.93 (1H, t, J=2.8 Hz), 2.26 (1H, ddd, J=2.8, 7.0, 16.8 Hz), 2.39 (1H, ddd, J=2.8, 4.0, 16.8 Hz), 3.97 (1H, ddd, J=4.0, 5.2, 6.7 Hz), 4.12 (1H, ddt, J=6.4, 6.7, 1.2 Hz), 5.09 (1H, dt, J=10.4, 1.2 Hz), 5.16 (1H, dt, J=17.1, 1.2 Hz), 5.75 (1H, ddd, J=6.1, 10.4, 17.1 Hz).
MS m/z : 382 (M^+), 367 (M^+-Me), 325 (M^+-tBu).

Reference Example 15

Synthesis of (3R,4R,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (40)

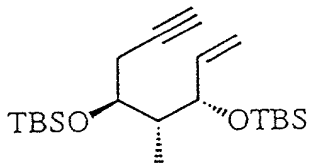


(40)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.01 (3H, s), 0.049 (3H, s), 0.051 (3H, s), 0.08 (3H, s), 0.89 (18H, s), 0.92 (3H, d, J=7.0 Hz), 1.86 (1H, dquin, J=4.0, 6.7 Hz), 1.95 (1H, t, J=2.8 Hz), 2.38 (2H, dd, J=2.7, 5.8 Hz), 3.88 (1H, ddd, J=4.0, 6.1, 6.4 Hz), 4.09 (1H, t, J=7.0 Hz), 5.10 (1H, dt, J=10.4, 1.5 Hz), 5.14 (1H, dt, J=17.4, 1.5 Hz), 5.81 (1H, ddd, J=7.0, 10.4, 17.4 Hz).
MS m/z : 382 (M^+), 367 (M^+-Me), 325 (M^+-tBu).

Reference Example 16

Synthesis of (3S,4R,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (41)



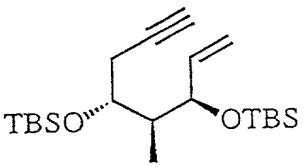
(41)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.03 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.76 (3H, d, J=7.0 Hz), 0.889 (9H, s), 0.892 (9H, s), 1.91 (1H, dquin, J=3.7, 7.0 Hz), 1.97 (1H, t, J=2.8 Hz), 2.36-2.40 (2H, m), 3.99-4.05 (2H, m), 5.09 (1H, dt, J=10.4, 0.9 Hz), 5.13 (1H, dt, J=17.1, 0.9 Hz), 5.73 (1H, ddd, J=7.6, 10.1, 17.1 Hz).

MS m/z : 382 (M^+), 367 (M^+-Me), 325 (M^+-tBu).

Reference Example 17

Synthesis of (3R,4S,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (42)



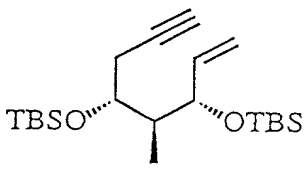
(42)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.03 (3H, s), 0.06 (3H, s), 0.07 (3H, s), 0.08 (3H, s), 0.76 (3H, d, J=7.0 Hz), 0.889 (9H, s), 0.891 (9H, s), 1.91 (1H, dquin, J=3.7, 7.0 Hz), 1.97 (1H, t, J=2.8 Hz), 2.31-2.43 (2H, m), 3.98-4.04 (2H, m), 5.10 (1H, dt, J=10.1, 1.5 Hz), 5.13 (1H, dt, J=17.1, 1.5 Hz), 5.74 (1H, ddd, J=7.6, 10.1, 17.1 Hz).

MS m/z: 382 (M^+), 367 (M^+-Me), 325 (M^+-tBu).

Reference Example 18

Synthesis of (3S,4S,5R)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (43)



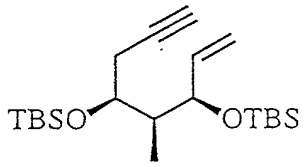
(43)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.01 (3H, s), 0.049 (3H, s), 0.051 (3H, s), 0.08 (3H, s), 0.89 (18H, s), 0.92 (3H, d, J=7.0 Hz), 1.85 (1H, dquin, J=3.7, 6.7 Hz), 1.96 (1H, t, J=2.8 Hz), 2.39 (2H, dd, J=2.8, 6.7 Hz), 3.88 (1H, ddd, J=4.0, 6.1, 6.4 Hz), 4.07 (1H, t, J=6.7 Hz), 5.10 (1H, dt, J=10.1, 1.8 Hz), 5.14 (1H, dt, J=18.3, 1.8 Hz), 5.81 (1H, ddd, J=7.0, 10.4, 17.4 Hz).

MS m/z : 367 (M^+-Me), 325 (M^+-tBu).

Reference Example 19

Synthesis of (3R,4S,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (44)



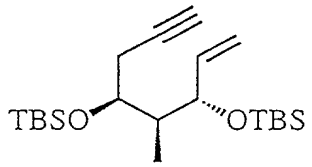
(44)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.01 (3H, s), 0.057 (3H, s), 0.063 (3H, s), 0.11 (3H, s), 0.78 (3H, d, J=7.0 Hz), 0.86 (9H, s), 0.90 (9H, s), 1.88 (1H, dquin, J=5.5, 6.7 Hz), 1.93 (1H, t, J=2.8 Hz), 2.26 (1H, ddd, J=2.8, 7.0, 16.8 Hz), 2.39 (1H, ddd, J=2.8, 4.0, 16.8 Hz), 3.97 (1H, dt, J=4.0, 5.5 Hz), 4.12 (1H, ddt, J=5.2, 6.7, 1.2 Hz), 5.09 (1H, dt, J=10.4, 1.2 Hz), 5.15 (1H, dt, J=17.1, 1.2 Hz), 5.75 (1H, ddd, J=6.7, 10.4, 17.4 Hz).

MS m/z : 382 (M^+), 367 (M^+-Me), 325 (M^+-tBu).

Reference Example 20

Synthesis of (3S,4S,5S)-3,5-bis(t-butyldimethylsilyloxy)-4-methyl-1-octen-7-yne (45)



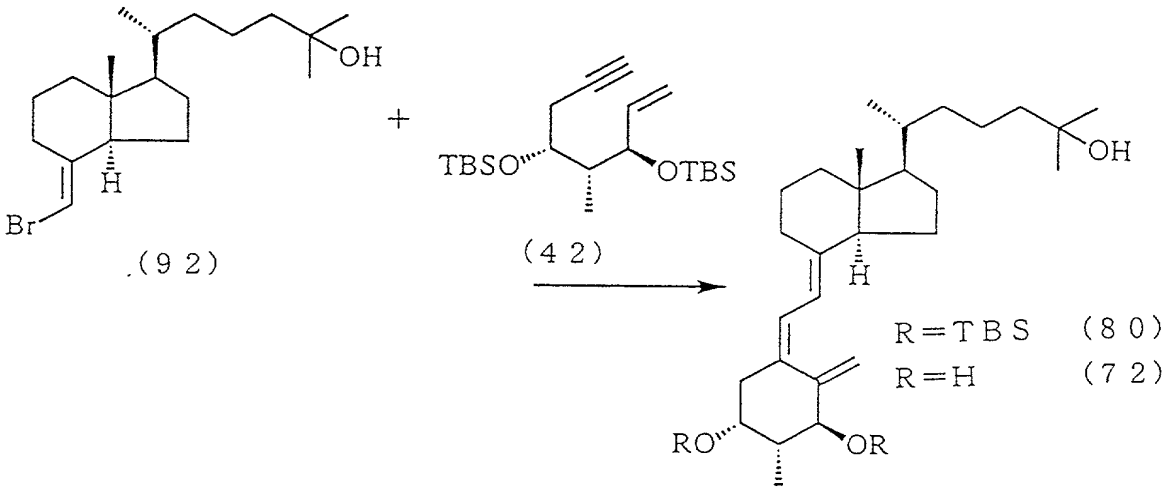
(45)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.01 (3H, s), 0.05 (3H, s), 0.07 (3H, s), 0.10 (3H, s), 0.88 (3H, d, J=7.0 Hz), 0.89 (9H, s), 0.90 (9H, s), 1.76-1.80 (1H, m), 1.93 (1H, t, J=2.8 Hz), 2.26 (1H, ddd, J=2.7, 7.0, 16.8 Hz), 2.40 (1H, ddd, J=2.7, 4.3, 16.8 Hz), 3.85 (1H, dt, J=7.0, 4.3 Hz), 4.11 (1H, ddt, J=5.8, 7.3, 1.8 Hz), 5.10 (1H, dt, J=10.1, 1.8 Hz), 5.14 (1H, dt, J=17.4, 1.8 Hz), 5.84 (1H, ddd, J=7.3, 10.1, 17.4 Hz).

MS m/z : 382 (M^+), 367 (M^+-Me), 325 (M^+-tBu).

Example 1

Synthesis of (20S)-1α,25-dihydroxy-2α-methyl-3β-vitamin D_3 (72)



An exo-methylene compound (92) (17 mg) was dissolved in 0.3 ml of toluene, and Et_3N (0.45 ml) was added to the resultant solution under argon atmosphere. $Pd_2(dba)_3 \cdot CHCl_3$ (1.9 mg, 0.03 equivalent) and Ph_3P (2.5 mg, 0.3 equivalent) were added, the resultant mixture was stirred at room temperature, then a toluene solution (0.2 ml) of the ene-yne compound (42) (13 mg, 0.7 equivalent) was added, and the mixture was stirred at room temperature for 10 min, and further made to react on an oil bath of 120℃ for 2.5 hr. After cooling, the reaction mixture was filtered, and the filtrate was purified by silica gel chromatography (AcOEt:hexane=1:3) to obtain a compound (80).

The obtained compound (80) was dissolved in 1 ml of methanol, CSA (11 mg, 1 equivalent) was added to the resultant solution, the mixture was made to react overnight at room temperature under argon atmosphere. The reaction mixture was evaporated, the residue was extracted with AcOEt after the addition of purified water. The extract was washed with brine, dried over magnesium sulfate and evaporated. The obtained crude product was purified by silica gel chromatography (AcOEt:hexane=1:1) and further by recycle-preparative HPLC (Lichrosorb RP-18, 70% MeCN/H_2O) to obtain colorless crystal (72) (9.3 mg, 6.3%).

^{1}H-NMR (400 MHz, $CDCl_3$-D_2O/TMS) δ : 0.53 (3H, s), 0.85 (3H, d, J=6.7 Hz), 1.08 (3H, d, J=6.8 Hz), 1.21 (6H, s), 1.12-2.04, (19H, m) 2.23 (1H, dd, J =7.9, 13.4 Hz), 2.67 (1H, dd, J=4.0, 13.4 Hz), 2.83 (1H ,m), 3.83 (1H, td, J =7.9, 4.0 Hz), 4.29 (1H, d, J=3.3 Hz), 5.01 (1H, d, J=1.8 Hz), 5.28 (1H, m), 6.01 (1H, d, J=11.3 Hz), 6.39 (1H, d, J=11.3 Hz).

UV (EtOH) λ max : 266 nm.

MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$).

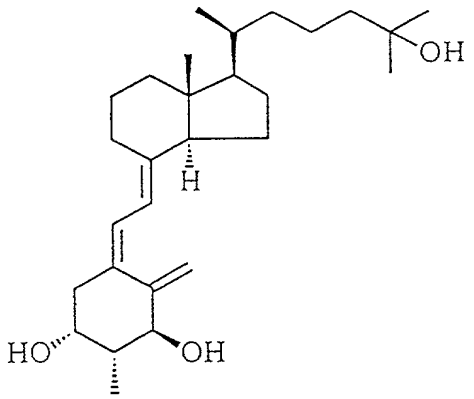
HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3443.

Using reaction conditions similar to Example 1, the following 1, 25-dihydroxy-2-methylvitamin D_3 derivatives were prepared.

Example 2

Synthesis of (20S)-1 α ,25-dihydroxy-2 β -methyl-3 β -vitamin D_3 (68)



(68)

^{1}H-NMR (400 MHz, $CDCl_3$-D_2O/TMS) δ : 0.55 (3H, s), 0.85 (3H, d, J=6.4 Hz), 1.15 (3H, d, J=6.7 Hz), 1.21 (6H, s), 1.17-2.01 (19H, m) 2.42 (1H, dd, J=13.9, 4.9 Hz), 2.52 (1H, d, J=13.9 Hz), 2.82 (1H, dd, J=11.9, 4.0 Hz), 3.99-4.04 (1H+1H, m), 5.02 (1H, t, J=1.8 Hz), 5.37 (1H, t, J=1.8 Hz), 6.03 (1H, d, J=11.3 Hz), 6.35 (1H, d, J=11.3 Hz).

UV(EtOH) λ max : 263 nm.

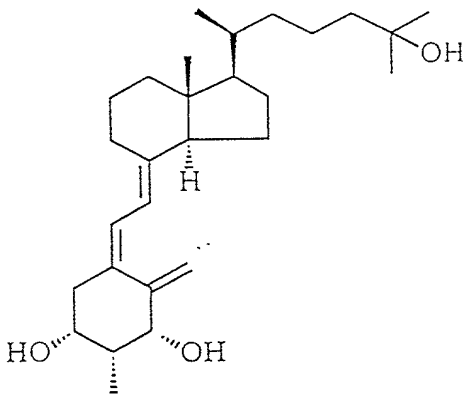
MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$).

HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3441.

Example 3

Synthesis of (20S)-1 β ,25-dihydroxy-2 β -methyl-3 β -vitamin D_3 (69)



(69)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.55 (3H, s), 0.85 (3H, d, J=6.7 Hz), 1.22 (6H, s), 1.23 (3H, d, J=7.3 Hz), 2.17 (1H, d, J=4.3 Hz), 2.50 (1H, brd, J=12.5 Hz), 2.59(1H, dd, J=14.0, 3.7 Hz), 2.79 (1H, d, J=7.6 Hz), 2.85 (1H, dd, J=12.5, 4.9 Hz), 3.91(1H, m), 4.17 (1H, m), 5.01 (1H, d, J=2.1 Hz), 5.25 (1H, d, J=1.8 Hz), 6.09 (1H, d, J=11.3 Hz), 6.48 (1H, d, J=11.3 Hz).

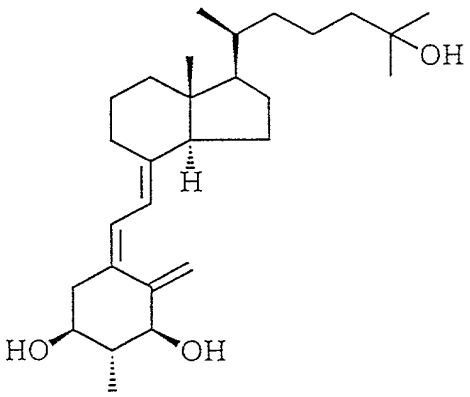
MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$), 379 (M^+-$2H_2O$-Me).

HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3446.

Example 4

Synthesis of (20S)-1α,25-dihydroxy-2β-methyl-3α-vitamin D_3 (70)



(70)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.54 (3H, s), 0.85 (3H, d, J=6.4 Hz), 1.06 (3H, d, J=7.0 Hz), 1.22 (6H, s), 2.12 (1H, d, J=2.8 Hz), 2.34 (1H, dd, J=14.7, 7.0 Hz) ,2.60 (1H, brs), 2.64 (1 H, dd, J=13.4, 2.8 Hz), 2.84 (1H, dd, J=11.6, 3.1 Hz), 3.65 (1H, m), 3.90(1H, m), 5.05 (1H, d, J=1.8 Hz), 5.30 (1H, d, J=2.7 Hz), 6.02 (1H, d, J=11.3 Hz), 6.41 (1H, d, J=11.3 Hz).

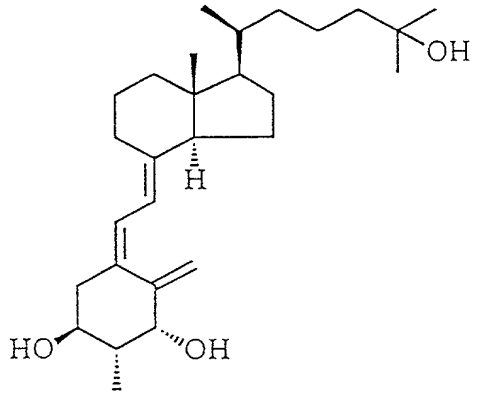
MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$), 379 (M^+-$2H_2O$-Me).

HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3447.

Example 5

Synthesis of (20S)-1β,25-dihydroxy-2β-methyl-3α-vitamin D_3 (71)



(71)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.54 (3H, s), 0.85 (3H, d, J=6.4 Hz), 1.10 (3H, d, J=6.7 Hz), 1.22 (6H, s), 1.68 (2H, m), 1.85 (2H, m), 1.98 (2H, m), 2.24 (1H, dd, J=13.4, 8.5 Hz), 2.65 (1H, dd, J=13.4, 4.3 Hz), 2.82 (1H, dd, J=12.2, 4.3 Hz), 3.81 (1H, m), 4.27 (1H, m), 5.02 (1H, d, J=2.1 Hz), 5.28 (1H, d, J=1.8 Hz), 6.02 (1H, d, J=11.3 Hz), 6.40 (1H, d, J=11.3 Hz).

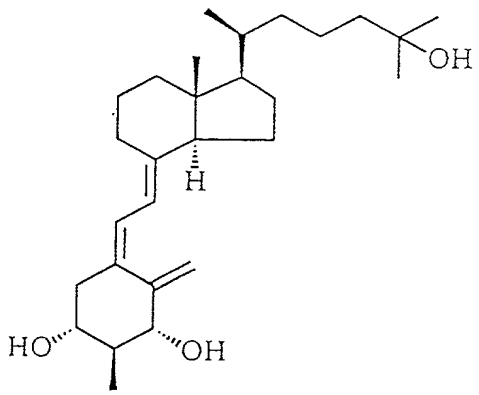
MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$), 379 (M^+-$2H_2O$-Me).

HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3446.

Example 6

Synthesis of (20S)-1β,25-dihydroxy-2α-methyl-3β-vitamin D_3 (73)



(73)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.55 (3H, s), 0.85 (3H, d, J=6.4 Hz), 1.02 (3H, d, J=7.0 Hz), 1.22 (6H, s), 1.83 (1H, m), 2.00 (2H, m), 2.11 (1H, m), 2.27 (1H, d, J=7.0 Hz), 2.34 (1H, dd, J=14.0, 5.5 Hz), 2.65 (1H, dd, J=14.0, 7.8 Hz), 2.84 (1H, dd, J=12.2, 4.3 Hz), 3.72 (1H, m), 3.97 (1H, t, J=4.9 Hz), 5.07 (1H, d, J=2.1 Hz), 5.30 (1H, d, J=2.1 Hz), 6.04 (1H, d, J=11.3 Hz), 6.43 (1H, d, J=11.3 Hz).

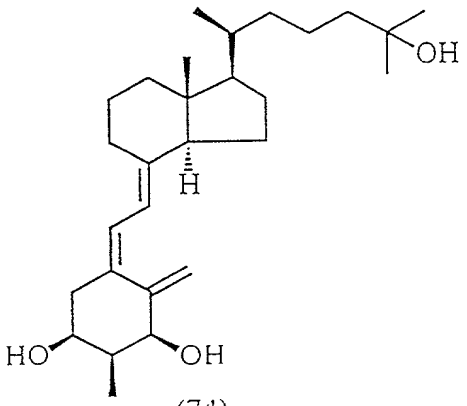
MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$), 379 (M^+-$2H_2O$-Me).

HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3445.

Example 7

Synthesis of (20S)-1α,25-dihydroxy-2α-methyl-3α-vitamin D_3 (74)



(74)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.53 (3H, s), 0.85 (3H, d, J=6.4 Hz), 1.21 (6H, s), 1.22 (3H, d, J=7.0 Hz), 2.09 (1H, d, J=4.6 Hz), 2.49 (1H, d, J=14.7 Hz), 2.58 (1H, dd, J=14.0, 3.7 Hz), 2.80 (1H, d, J=7.9 Hz), 2.85 (1H, m), 3.91 (1H, m), 4.17 (1H, m), 4.98 (1H, d, J=2.1 Hz), 5.23 (1H, d, J=1.8 Hz), 6.03 (1H, d, J=11.3 Hz), 6.48 (1H, d, J=11.3 Hz).

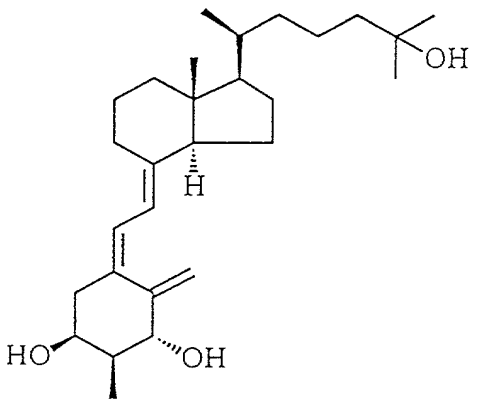
MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$), 379 (M^+-$2H_2O$-Me).

HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3447.

Example 8

Synthesis of (20S)-1 β ,25-dihydroxy-2 α -methyl-3 α -vitamin D_3 (75)



(75)

^{1}H-NMR (400 MHz, $CDCl_3$/TMS) δ : 0.53 (3H, s), 0.85 (3H, d, J=6.4 Hz), 1.13 (3H, d, J=6.7 Hz), 1.21 (6H, s), 1.69 (2H, m), 1.84 (2H, m), 1.98 (2H, m), 2.41 (1H, dd, J=13.7, 5.5 Hz), 2.51 (1H, dd, J=13.4, 2.4 Hz), 2.82 (1H, m), 4.02-4.08 (2H, m), 5.01 (1H, d, J=1.8 Hz), 5.35 (1H, d, J=1.8 Hz), 6.01 (1H, d, J=11.6 Hz), 6.36 (1H, d, J=11.6 Hz).

MS m/z : 430 (M^+), 412 (M^+-H_2O), 394 (M^+-$2H_2O$), 379 (M^+-$2H_2O$-Me).

HR-MS, calculated for $C_{28}H_{46}O_3$: 430.3447,

found : 430.3445.

Example 9

Binding affinities of objective compounds of the present invention for a bovine thymus 1 α ,25-dihydroxyvitamin D_3 receptor (VDR)

The content (about 25 mg) of an ampule of a bovine thymus vitamin D receptor kit made by Yamasa Shoyu Ltd. was dissolved in 55 ml of a 0.05 M phosphoric acid-0.5 M potassium buffer solution (pH 7.4). Fifty μl of the ethanol solution of a compound to be tested and 500 μl of the receptor solution were pre-incubated at room temperature for 1 hr. To the treated mixture, 50 μl of a [26,27-methyl-^{3}H]1 α ,25-dihydroxyvitamin D_3 solution (131 Ci/mmol, 16,000 dpm) was added so that the final concentration became 0.1 nM, and the resultant mixture was incubated overnight at 4℃. Both of

the bound and the non-bound [26,27-methyl-^{3}H]1α,25-dihydroxy-vitamins D_3 were subjected to centrifugation after the addition of 200 μl of dextran-coated charcoal. To 500 μl of the supernatant, 9.5 ml of a liquid scintillation cocktail (ACS-II) was added, and the radioactivity of the resultant mixture was measured by a liquid scintillation counter.

The binding affinity of a compound to be tested for the D_3-receptor (VDR) was expressed by a relative intensity ratio based on 100 for 1α, 25-dihydroxyvitamin D_3 by determining the concentration which inhibits the binding of [26,27-methyl-^{3}H]1α,25-dihydroxy-vitamin D_3 by 50%. The results are shown in the following Table.

Compound	Binding Affinity for VDR	Compound	Binding Affinity for VDR
1α,25-$(OH)_2VD_3$	100		
Compound (65)	13	Compound (68)	160
Compound (1)	0.05	Compound (69)	0.03
Compound (2)	0.3	Compound (70)	0.08
Compound (3)	0.8	Compound (71)	7
Compound (4)	400	Compound (72)	1200
Compound (5)	0.05	Compound (73)	0.05
Compound (6)	4	Compound (74)	17
Compound (7)	0.06	Compound (75)	0.03

Herein, the compounds (1) to (7) and the compound (65) shown in the above table are reference examples, and are expressed by the followings, respectively.

(20R)-1β,25-dihydroxy-2β-methyl-3β vitamin D_3,

(20R)-1α,25-dihydroxy-2β-methyl-3α vitamin D_3,

(20R)-1β,25-dihydroxy-2β-methyl-3α vitamin D_3,

(20R)-1α,25-dihydroxy-2α-methyl-3β vitamin D_3,

(20R)-1β,25-dihydroxy-2α-methyl-3β vitamin D_3,

(20R)-1α,25-dihydroxy-2α-methyl-3α vitamin D_3,

(20R)-1β,25-dihydroxy-2α-methyl-3α vitamin D_3 and

(20R)-1 α,25-dihydroxy-2 β-methyl-3 β vitamin D_3.

Example 10

Activities of objective compounds of the present invention on differentiation induction effect of HL-60 cell

HL-60 cell was purchased from a cell bank (Japanese Cancer Research Resource Bank, Cell Number: JCRB 0085). The cell was stored as a frozen storage stock for preventing the change of cell characteristics attributable to successive cultivation. Before the initiation of experiments, the cell was defrosted, and successive cultivation was started for using the cell in the experiments. The cell which had been treated by successive culturing for about one to six months was used for the experiments. The successive culturing was carried out by centrifugally recovering cells from cultivation mixture in suspension culture, and diluting the collected cell concentrate in a fresh culture medium at a ratio of about 1/100 ($1\text{-}2\times10^5$ cells/ml). As the culture medium, an RPMI-1640 medium containing 10% fetal bovine serum was used. Successively cultured cells were centrifugally collected, and they were dispersed in a culture medium at the concentration of 2×10^4 cells/ml. The dispersion was seeded into a 24-well culture petri dish at 1 ml/well. An ethanol solution (1×10^{-9}M to 1×10^{-6}M) of a compound of the present invention was added to this system at 1 μl/well. Further, regarding 1 α,25$(OH)_2D_3$, an ethanol solution of 1×10^{-7} M to 1×10^{-4} M was added at 1 μl/well, and for the control, ethanol was added at 1 μl/well. After culturing at 37℃ for 4 days under a 5% CO_2 atmosphere, the cells were centrifugally collected. Nitroblue tetrazolium (NBT) reduction activity was determined as follows. That is, the collected cells were suspended in a fresh culture medium, and NBT and 12-O-tetradecanoylphorbol-13-acetate were added to the resultant suspension so that their concentrations became 0.1% and 100 nM, respectively. After the mixed suspension was incubated at 37℃ for 25 min, a cytospin sample was prepared. After air drying, it was stained with Kernechtrot, and the ratio of the positive cells of NBT reduction activity was determined under an optical microscope. The results are shown in the following Table.

Activities of Compounds of the Present Invention on Nitroblue Tetrazolium Reduction Activity in HL-60 Cell

Compound	Concentration (M)	Positive cells (%) of nitroblue tetrazolium reduction activity
Control		1.5
1 α ,25-$(OH)_2D_3$	10^{-10}	4.3±1.2
	10^{-9}	36.8±2.0
	10^{-8}	86.1±2.6
	10^{-7}	96.5±1.0
Compound (68)	10^{-12}	1.7±0.3
	10^{-11}	2.8±0.7
	10^{-10}	57.7±5.0
	10^{-9}	95.7±1.0
Compound (71)	10^{-12}	1.5±0.8
	10^{-11}	1.8±0.8
	10^{-10}	2.0±1.0
	10^{-9}	40.5±1.8
Compound (74)	10^{-12}	6.4±1.1
	10^{-11}	17.0±2.3
	10^{-10}	16.7±1.1
	10^{-9}	96.4±1.4
Compound (72)	10^{-12}	3.7±0.8
	10^{-11}	94.4±1.8
	10^{-10}	95.7±2.3
	10^{-9}	96.2±2.0

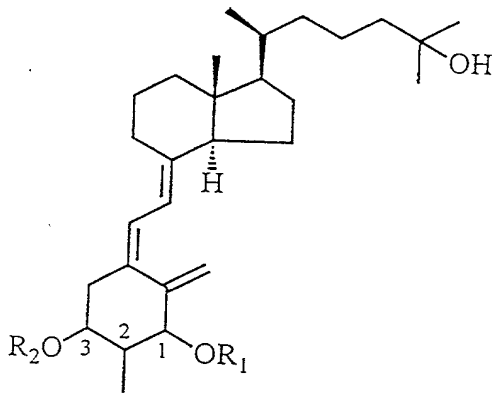
Industrial Field of Application

Each of 1, 25-dihydroxy-2-methylvitamin D_3 derivatives expressed the above formula (I) provided by the present invention can be effectively used for diseases (osteoporosis, rickets, hyperthyroidism, etc.) for which the usefulness of vitamin D_3 derivatives are widely recognized. Among these diseases, compounds of the present invention are especially effective for diseases (tumors, psoriasis, etc.) attributable to cell differentiation failure owing to extremely strong differentiation-inducing activity.

Further, each of 1, 25-dihydroxy-2-methylvitamin D_3 derivatives exhibits different affinities for between a vitamin D receptor and a vitamin D binding protein depending on the kind of stereoisomer derived from the 1-, 2- and 3-positions, in such a manner that a stereoisomer has high affinity for the vitamin D receptor and at the same time high affinity for the vitamin D binding protein, and another isomer has a high affinity for the vitamin D receptor but a low affinity for the vitamin D binding protein. The derivatives therefore are useful as treating agents for vitamin D metabolic disorders suitably corresponding to characteristics of action of the derivatives.

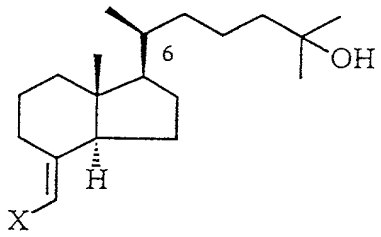
CLAIMS

1. 1, 25-dihydroxy-2-methylvitamin D3 derivatives expressed by the following general formula (I),

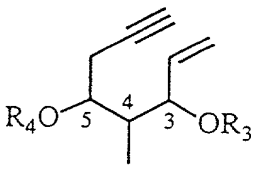


[wherein each of R_1 and R_2 is independently a hydrogen atom or a tri(C_1 to C_7 alkyl)silyl group; herein configurations of asymmetric carbons at the 1-, 2- and 3-positions are each independently α-configuration or β-configuration].

2. A method for producing a vitamin D_3 derivative described in the claim 1, wherein an exo-methylene compound expressed by the following general formula (II),



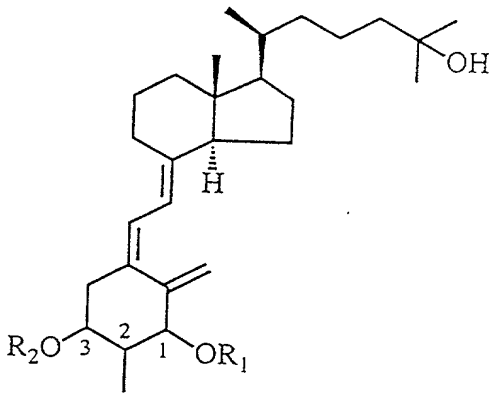
(wherein X is a bromine atom or an iodine atom) is made to react with an ene-yne compound expressed by the following general formula (III),



[wherein R_3 and R_4 are each independently a hydrogen atom or a tri(C_1 to C_7 hydrocarbon)silyl group] in the presence of a palladium catalyst, and optionally the protecting group of the tri(C_1 to C_7 hydrocarbon)silyl group is removed.

ABSTRACT

Provided are 1, 25-dihydroxy-2-methylvitamin D3 derivatives expressed by the general formula (I),



[wherein each of R_1 and R_2 is independently a hydrogen atom or a tri(C_1 to C_7 alkyl)silyl group; herein configurations of asymmetric carbons at the 1-, 2- and 3-positions are each independently α-configuration or β-configuration] and their production methods.

The compound is useful as a treating agent for osteoporosis, rickets, hyperthyroidism, etc.

Declaration and Power of Attorney for Patent Application

特許出願宣言書

Japanese Language Declaration

私は、下欄に氏名を記載した発明として、以下の通り宣言する：

As a below named inventor, I hereby declare that:

私の住所、郵便の宛先および国籍は、下欄に氏名に続いて記載したとおりであり、

My residence, post office address and citizenship are as stated below next to my name,

名称の発明に関し、請求の範囲に記載した特許を求める主題の本来の、最初にして唯一の発明者である（一人の氏名のみが下欄に記載されている場合）か、もしくは本来の、最初にして共同の発明者である（複数の氏名が下欄に記載されている場合）と信じ、

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

"VITAMIN D_3 DERIVATIVE AND ITS PRODUCTION METHOD"

その明細書を
（該当するほうに印を付す）

the specification of which
(check one)

☐ ここに添付する。

☐ is attached hereto.

☐ _____ 日に出願番号

☒ was filed on April 30, 1998 as

第 _____ 号として提出し、

International Application Serial No. PCT/JP98/01979

_____ 日に補正した。
（該当する場合）

and was amended on _____
(if applicable)

私は、前記のとおり補正した請求の範囲を含む前記明細書の内容を検討し、理解したことを陳述する。

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37部第1章第56条(a)項に従い、本願の審査に所要の情報を開示すべき義務を有することを認める。

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

Japanese Language Declaration

私は、合衆国法典第35部第119条、第172条、又は第365条に基づく下記の外国特許出願又は発明者証出願の外国優先権利益を主張し、さらに優先権の主張に係わる基礎出願の出願日前の出願日を有する外国特許出願又は発明者証出願を以下に明記する。

I hereby claim foreign priority benefits under Title 35, United States Code §119, §172 or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior foreign applications
先の外国出願

(Number) (番 号)	(Country) (国 名)	(Day/Month/Year Filed) (出願の年月日)	Priority claimed 優先権の主張 Yes あり	No なし
9-114695	Japan	02/May/1997	☒	☐
			☐	☐
			☐	☐
			☐	☐
			☐	☐

私は、合衆国法典第35部第120条に基づく下記の合衆国特許出願の利益を主張し、本願の請求の範囲各項に記載の主題が合衆国法典第35部第112条第1項に規定の態様で先の合衆国出願に開示されていない限度において、先の出願の出願日と本願の国内出願日又はＰＣＴ国際出願日の間に公表された連邦規則法典第37部第1章第56条(a)項に記載の所要の情報を開示すべき義務を有することを認める。

I hereby claim the benefit of Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose any material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (出願番号)	(Filing Date) (出願日)	(現 況) 特許済み、係属中、放棄済み	(Status) (patended, pending abandoned)

私は、ここに自己の知識に基づいて行った陳述がすべて真実であり、自己の有する情報及び信ずるところに従って行った陳述が真実であると信じ、更に故意に虚偽の陳述等を行った場合、合衆国法典第18部第1001条により、罰金もしくは禁固に処せられるか、又はこれらの刑が併科され、又はかかる故意による虚偽の陳述が本願ないし本願に対して付与される特許の有効性を損なうことがあることを認識して、以上の陳述を行ったことを宣言する。

I hereby declare that all statements made herein of my own knowledge are true; and further that all statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Japanese Language Declaration

委任状： 私は、下記発明者として、以下の代理人をここに選任し、本願の手続きを遂行すること並びにこれに関する一切の行為を特許商標局に対して行うことを委任する。
（代理人氏名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith *(list name and registration number)*

I hereby appoint John H. Mion, Reg. No. ~~18,879~~ 18,879; Donald E. Zinn, Reg. No. 19,046; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. ~~21,092~~ 21,092; Darryl Mexic, Reg. No. ~~23,063~~ 23,063; Robert V. Sloan, Reg. No. ~~22,775~~ 22,775; Peter D. Olexy, Reg. No. ~~24,513~~ 24,513; J. Frank Osha, Reg. No. ~~24,625~~ 24,625; Waddell A. Biggart, Reg. No. 24,861; Robert G. McMorrow, Reg. No. 19,093; Louis Gubinsky, Reg. No. ~~24,835~~ 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. ~~26,916~~ 26,916; Joseph J. Ruch, Jr., Reg. No. ~~26,577~~ 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. ~~25,665~~ 25,665; Alan J. Kasper, Reg. No. ~~25,426~~ 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. ~~30,764~~ 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562; Brian W. Hannon, Reg. No. 32,778; Abraham J. Rosner, Reg. No. ~~33,276~~ 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. ~~33,102~~ 33,102; and Brett S. Sylvester, Reg. No. 32,765, my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and request that all correspondence about the application be addressed to ~~SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC, 2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037-3202.~~

(3)

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2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037

直通電話連絡先： （名称及び電話番号） Direct Telephone Calls to: *(name and telephone number)*

(202)293-7060

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	Sagamiko-machi, Tsukui-gun, KANAGAWA 199-0106, JAPAN
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同第二発明者の署名 日付	Second inventor's signature Date Katsuhiro Konno Jan 17, 1999
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国籍	Citizenship Japanese JPY
郵便の宛先	Post office address c/o Faculty of Pharmaceutical Sciences, Teikyo University, 1091-1, Suarashi,
	Sagamiko-machi, Tsukui-gun, KANAGAWA 199-0106, JAPAN

（第三又はそれ以降の共同発明者に対しても同様な情報および署名を提供すること。）

(Supply similar information and signature for third and subsequent joint inventors.)

第三の共同発明者の氏名（該当する場合）	Full name of third joint inventor, if any Toshie FUJISHIMA
同第三発明者の署名　日付	Third inventor's signature　Date Toshie Fujishima　Dec. 10, 1998
住所	Residence Tsujui-gun, ~~KANAGAWA,~~ JAPAN
国籍	Citizenship Japanese　JPX
郵便の宛先	Post office address c/o Faculty of Pharmaceutical Sciences, Teikyo University, 1091-1, Suarashi,
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第四の共同発明者の氏名（該当する場合）	Full name of fourth joint inventor, if any
同第四発明者の署名　日付	Fourth inventor's signature　Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post office address
第五の共同発明者の氏名（該当する場合）	Full name of fifth joint inventor, if any
同第五発明者の署名　日付	Fifth inventor's signature　Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post office address
第六の共同発明者の氏名（該当する場合）	Full name of sixth joint inventor, if any
同第六発明者の署名　日付	Sixth inventor's signature　Date
住所	Residence
国籍	Citizenship
郵便の宛先	Post office address